THE WORTH OF THE INDIVIDUAL IN MODERN SOCIETY

THE annual reports of successive presidents of the Carnegie Institution of Washington have earned a high reputation for the prescience and imagination with which they survey the varied aspects of the inter-relations of science and society and the contribution which the independent research institution can make. Nor are such problems surveyed from a narrow or nationalistic point of view, and it is not surprising that these reports are read with as much appreciation by British as by American readers, or that there is frequently to be discerned a striking parallelism between the trend of thought in the president's report and that disclosed in such current addresses of British men of science as the anniversary address to the Royal Society, a Reith Lecture, or a university lecture like the Fawley Foundation Lecture at the University of Southampton. Dr. Caryl P. Haskins's current report* is no exception, and its opening section, in which he considers the challenge which is offered to men and nations by times of severe stress, of profound change and radical adjustment, such as those in which we live, may profitably be considered alongside some passages in Lord Adrian's recent Fawley Lecture, "The Risks of Progress".

Dr. Haskins begins by pointing out that hazard is not the only, or even the dominant, quality of such times, which offer far more than mere tests of ingenuity in living to be met by essentially conventional, if strenuous, feats of adaptation and accommodation. If met with boldness, with balance and dedication, and above all with independence of mind and spirit, such times may also be times of unmatched opportunities for new orders of growth and self-realization, the ultimate benefits of which could far outweigh the short-term perils that pre-occupy and shadow our generation. Earlier generations living in times of comparable stress and change recognized the uncertainty and danger of their lives, but they realized also the enormous challenge of their days. To-day a new eagerness and audacity can be sensed, and a flavour of the immense significance of individual effort and achievement has come forward to our day. Each period is coloured by a certain dynamic fluidity—a special ability, possibly a special eagerness, to embrace the condition of change for its own sake, regardless of its specific content—and all seem to have attached a higher value to the dynamic process of living in their society than to the material consequences. New ideas were valued and exciting, and their communication was as important as their genesis. Exploration, or the search for innovation in one or another realm of experience, was specially valued.

No patterns, of course, as Dr. Haskins reminds us, are ever precisely repeatable, and although it is not surprising that we should find in our own age certain parallels with other ages which have been characterized, in the perspective of history, as creative or successful, in certain ways, he rightly adds, our challenge differs from that confronting earlier periods so greatly in degree as perhaps to be of another kind. In no earlier society can the challenge to acuity of comprehension, to specialized skills, to generalized insight, to the fullest development of individual capability in every field, have been so imperative, nor can the demand and the opportunity for individual development have approached those of our own day. In our age surely the stimulus, the demand and the opportunities for innovation, for individual creativeness, have reached unprecedented heights.

Dr. Haskins next directs attention to a qualitative difference in the practical technical demands of our times. Earlier dynamic ages have probably put first in their technical strategy the search for sources of physical power. Now the very success of that quest suggests that in future other aspects of our conquest of Nature, concerned more particularly with understanding and control, will assume a more conspicuous place in our technology, as is already happening. In the future, they must emphasize further and give a keener incentive to a broader, perhaps subtler and deeper comprehension of Nature as a practical requirement of living.

Those observations point to the vital importance of education in the world to-day and to the soundness of the philosophy displayed in the first part of the recent Crowther Report. Dr. Haskins goes on to bring this out even more clearly when he shows that for nations, as for men, times of unusual stress and challenge are a supreme test, not alone of effectiveness in action, but also and primarily of inner cohesion and verve, of inner wholeness. The severest practical challenge can be met successfully by a people only in its own characteristic fashion, consonant with its own most deeply held values. Unless these are adequate and are undegraded, the whole strength and balance of a nation will be inadequate.

We are not, Dr. Haskins reminds us, immune from the dangers which swiftly eroded some of the great ages of the past. We are faced by practical technical challenges of a national and an international character in which the search for knowledge and understanding of Nature for its own sake is often linked with material achievements indispensable to our prosperity, or even essential for survival. In such a situation it is easy to mistake the end of the road for the road itself, and it is imperative accordingly that we should be clear about our deepest motivations, that we meet these challenges in our own way and by courses that will reinforce our own ethic. Whether we are individually directly concerned or not with scientific matters, we must comprehend the values that underlie the search for scientific verities and appreciate how vividly they mirror our more generalized ethic.

* The Report of the President of the Carnegie Institution of Washington, 1958–1959. Pp. 54. (Reprinted from *Carnegie Institution of Washington Year Book* 58, for the year 1958–1959.) (Washington, D.C.: Carnegie Institution of Washington, 1959.)

The three points of anchorage which Dr. Haskins picks out from the philosophy of American society for special emphasis are no less part of the philosophy and tradition of Britain. First, there is that primary dedication to the individual and to his creativeness, that over-riding commitment which reveals the national stature primarily as a function of the welfare and perfection of realization of the worth and stature of the individual. This, Dr. Haskins writes, has been America's particular national pride to cultivate and foster, and on it depend the fundamental orientation and integrity of her society.

The second and third, like the first, may equally be regarded as part of America's heritage from Britain. The basically religious culture of America is closely linked with the first element, and though the dedication to the individual may be identified too narrowly with the Calvinist tradition from New England, it is undoubtedly largely derived from roots that are not materialistic. The third well-spring is a deep respect for, and concern with, both learning and innovation, and especially the exploration of Nature. The American people have traditionally loved the challenge of technical change; but equally the search for natural knowledge purely for its own sake is deeply indigenous in American culture.

Dr. Haskins recognizes, of course, that in doing this science does no more than any other creative intellectual activity; but nevertheless, in its dedication of individual effort, its primary seeking after universally communicable knowledge of the natural world, in the discipline of its economy and its parsimony, and in its sense of style, scientific research must be reckoned to-day as one of the essential bulwarks of the deepest national values of the United States. That is largely true of other Western democracies, and in those democracies also material success offers a strong and continuing threat to such a non-materialistic ethic. It is not only in the United States that society needs a more explicit image of the real nature of its deepest motives—an image to which science may have an important part to contribute.

At this point Dr. Haskins touches on the problem of the two cultures as outlined by Sir Charles Snow. Whether or not this gulf arises from the confusion of application with creation—of regarding the scientific process as primarily an ever-expanding fount of technical product—the most serious danger is not in the field of education or communication, but rather that the divergence of experience may set and harden a real and fundamental dichotomy of attitudes and values between the two worlds, and encourage an unbridgeable hostility between them. The threat of such a fundamental division between the scientific and non-scientific world arises primarily from the divergence or misunderstanding of values rather than from unfamiliarity of subject-matter. Such a sense of division is inherently dangerous, and Dr. Haskins emphasizes some of the more serious hazards.

First, noting the consistent exponential growth of fundamental research since the time of Newton, doubling in volume approximately every ten or fifteen years, and at an appreciably greater rate than many other activities, he suggests that such a range and sweep of scientific effort must involve not merely the re-making of our culture materially but also the depletion of other creative activities in art, literature and humanism. This, of course, is one of the aspects of university expansion, particularly with regard to scientists and technologists, which already causes much concern in academic circles and elsewhere. If the gulf between the realms of science and other departments of human activity is indeed unbridgeable, involving irreconcilable differences of value and motive, the position is serious, for it implies that science must inevitably be permanently competitive with other areas of human effort.

While this could reduce the multivariant range of skills and interests which society needs to-day—and must indeed seek to encourage and expand—Dr. Haskins can point to evidence on the other side. Good minds in science, for example, tend also to be naturally good minds in other fields, and often to seek spontaneously the common denominators among them. Again, some of the most impressive scientific disciplines of to-day can be traced back historically in an almost unbroken evolution of ideas to representations of beauty and order in Nature on the part of eighteenth-century observers, in a pattern which to-day we might characterize as more artistic than scientific.

Even more significantly, perhaps, Dr. Haskins refers to the way in which, one by one, over the years, the barriers of attitude, of ways of thinking and modes of attack which, since the time of Newton, have traditionally segregated the concerns of science, put them in contrast with, and set them apart as narrower and somewhat different from, those of the rest of the world, are, in the larger philosophical sense, being penetrated. Post-Newtonian scientists knew, or soon discovered, that they must restrict their framework of scientific investigation to a limited segment of the world if their methods were to be applied successfully, and they ultimately paid with a highly disciplined parochialism for added depth and penetration; the notion of an unbridgeable dichotomy between scientific and extra-scientific orientation and concerns may be in part a consequence. A dramatic widening of the areas of scientific concern, with a progressive lowering of old barriers, however, is one of the major characteristics of our age, and it is conceivable that our notions about the separateness of the concerns and the domain of science from the rest of the world may become further blurred and ultimately fade.

When and if that occurs, it will come about, not by one view of the world replacing another, but rather by a convergence on common interests and a common kingdom. Such a development may prove to be the most powerful of all levellers of barriers, the most effective of any bridges spanning the gulf between the scientific and non-scientific points of view in the years to come. Unlike Lord Adrian, Dr. Haskins is unconcerned by the way in which in our time academic science and its relation to technology and industry have been violently forced out of the pattern which Huxley prescribed for science in the

Victorian period. The risks of progress at which he looks are intellectual rather than physical, and he believes that our concern that the exponential growth of scientific effort may disastrously pre-empt our human resources of mind and spirit may prove to be largely irrelevant.

Uncommitted scientific research, however, has a broader and deeper task than the winning of new knowledge, and apart from its contribution to essential human values, within its framework of opportunities, its breadth of horizon, its range of concepts and of problems, it has an important general obligation to discharge. In a vast technological society such as ours, bigness rather than success threatens the sanctity and maintained sense of worth of the individual. Survival depends on skilfully and successfully marshalling the work of myriads of individuals into the achievements of organized groups, and it is far too easy to forget that the very essence of creativeness must lie with the individual as contrasted with the group; the greatest strength of the group may be, at another time and in another place, to develop the full consequences of the vision of the individual.

That, Dr. Haskins insists, in conclusion, is what the research environment must stand for. To provide for the probity and the freedom of choice and action, to furnish the dynamic facilities for broad contacts and wide communication; and simultaneously, the shielding and opportunity for isolation that are so important to innovation and so precious at its most sensitive and valuable point; to foster flexibility and growth in the disciplines with which it is concerned—these are important and essential tasks. Yet more widely and profoundly significant, however, is its duty of manifesting its spirit as a society devoted to and sustained by the earned dignity of the individuals composing it. This is indeed the image of our larger society, which, as that society must be constantly reminded, alone can give it meaning, autonomy and authority. However successfully such a society may avert the physical risks on which Lord Adrian touched, it will not long endure unless it looks within and ensures that spiritual renewal and maintenance of values for which Dr. Haskins pleads so earnestly and well.

ZOOLOGICAL BORDERLANDS

On the Track of Unknown Animals

By Dr. Bernard Heuvelmans. Translated from the French by Richard Garnett. Pp. 558+52 photographs. (London: Rupert Hart-Davis, Ltd., 1959.) 35*s*. net.

BOOKS, like fashions, seem to follow cycles. The past decade has seen the appearance of several works devoted largely to speculation on the identity of mysterious unknown animals, descriptions of 'living fossils', and accounts of the more striking of the zoological discoveries of the present and past century. Possibly the sensational discovery of *Latimeria* in 1938, and the interest aroused by reports of unknown ape-like creatures in the Himalayas, have helped to popularize such topical books. In his preface, Dr. Heuvelmans has acknowledged his indebtedness to earlier writers in the same field; but it must be acknowledged that his is the most comprehensive, the best written, and certainly the most challenging work of its kind. The amount of research, as indicated by the extensive bibliography, must have been enormous, ranging from early classical writers to modern travellers and naturalists. Whether Dr. Heuvelmans is discussing the legendary Nittaewo (the lost people of Ceylon), the abominable snowman himself, or the supposed great aquatic reptile of Central Africa, his net is spread far and wide to bring in all available accounts and evidence. His ingenuity in linking legends, native stories or hunters' tales to produce a composite picture of some animal not yet recognized by science is praiseworthy, and certainly produces a great deal of engrossing reading matter.

But enthusiasm for the subject, allied to a fertile imagination, has resulted in a certain one-sidedness of approach, and many of his suggestions and conclusions, ingenious as they may be, lead to a feeling that the reverse of the medal has not been shown, or has been too briefly mentioned. It is the author's expressed conviction that some at least of the larger animals believed to have been long extinct are still surviving, and that there are also still unidentified large creatures, known only from native reports, or other hearsay evidence, concealed in the less well explored regions of the Earth. The many important zoological discoveries of the past hundred years or more are, in his view, only an indication of many more yet to come, rather than the natural consequence of the rapid opening up of so many of the wilder places of the Earth during that period.

It is in the development and illustration of this theme that the author allows his antipathy to the professional zoologist to colour all his views and warp his judgment. It appears that the professional zoologist, almost without exception, has resolutely turned a deaf ear to all reports of unknown animals, and has stubbornly refused to consider the possible existence of any creature the bones or skin of which are not on the museum table. In defence of the maligned professional, it should not be necessary to point out that it is only in relation to the established and known that the unestablished and unknown can be considered, and that the essential reference works and catalogues listing the multitudinous life around us are largely the work of the professional systematists. The accusation that zoologists are more concerned with adding names to the literature on insufficient grounds comes ill from a writer who, in this book, proposes three new specific names and one new generic name on the most tenuous of evidence, while ignoring most of the established rules of taxonomic procedure. Dr. Heuvelmans defends his proposal of the new name *Dinanthropoides nivalis* (p. 178) for the abominable snowman (the evidence being photographs of tracks in the snow and photographs of a scalp not proved to be associated with the animal making the tracks) on the incorrect ground that "this procedure is not only universal in palæontology, but happens fairly often in zoology". In support, he quotes the first description of the giant panda, the alleged Andean wolf, the takahe (*Notornis*), the pygmy hippopotamus and the okapi. In actual fact none of these were named until tangible specimens, whether skins or skulls, were examined and compared.

This bias against the professional leads Dr. Heuvelmans to turn for support for his views to writers

about whose reliability and status there can be widely differing opinions. Even the introduction by the well-known author and collector of live animals, Gerald Durrell, is coloured by the same bias. Incidentally the latter's statement that the okapi was known only as an extinct relative of the giraffe until it was discovered living is quite inaccurate, and his reference to the 'surprising' discovery of the king cheetah in Southern Rhodesia is less convincing when it is known that the animal is no more than a colour mutation of the common cheetah.

In a work of this scope and length there are inevitably a number of errors and inaccuracies. A more careful checking of references would have avoided many of these. But the most serious charge that can be made is the over-emphasis on hearsay and unsupported tales which are quoted as evidence for the existence of some hypothetical animal, and the arrangement of factual evidence to support a case. For example, Fig. 81 (p. 314) is a sketch, full face, of the head of the alleged South American anthropoid or Loy's monkey, based on the photograph which is the only material evidence. In the photograph of the dead monkey the jaw sags, resulting in an unnatural appearance. In the sketch, shown with mouth shut, the face is compared with a reconstruction by von Koenigswald, 1938, of the face of *Pithecanthropus*. We are then told that if one disregards the many features in the *Pithecanthropus* face which cannot be inferred from the bones, and must therefore have been invented, the shape of the face is the same in the two. The author continues—"Although it [Loy's monkey] has been photographed full face, one can see that its profile is more like that of a capuchin monkey" (rather than a spider monkey, with which this animal is identified by primate specialists). Such ingenious presentation of evidence does not give one a great deal of faith in Dr. Heuvelmans' ability to hold a fair balance.

Again, in the case of the king cheetah (pp. 419–420), the description by R. I. Pocock in 1926 of what was thought at the time to be a very distinct new species is used as an illustration of how unreported animals can survive in a settled area for many years without discovery. But Dr. Heuvelmans does not tell the reader that in 1939 Pocock published his own conviction that this was not a new species after all, but simply a pattern mutation of the common cheetah. An illustration of the printing at full length of stories which eventually prove to have a prosaic ending is found in Chapter 20, devoted to the possibility of pterodactyls and other prehistoric reptiles still existing in Africa. Ivan T. Sanderson's vivid account of an encounter with a great black flying creature in the Cameroons (p. 491) is followed by a long discussion of its possible identity. But the footnote (p. 494) in which Sanderson is quoted (*in litt.*) as saying that the 'thing' was a bat, reduces the value of this contribution to nil.

Misquotations are frequent. The water civet, *Osbornictis piscivora* (p. 64), was discovered in the Ituri Forest not by Glover Allen in 1919 but by the collectors Lang and Chapin (of Congo peacock fame) in 1913, and it was Dr. J. A. Allen who described it in 1919. A skin and skull purporting to belong to a Nandi bear sent to the British Museum (Natural History) came not from Nyasaland (pp. 399–400) but from Uganda, hence Dr. Heuvelmans' comment on the unlikely locality has no validity. Again, we are told at the conclusion of the Nandi bear section (p. 414) that "Ivan T. Sanderson says that in the British Museum of Natural History there is a piece of fur [*sic*—'peau' in the original French edition] covered with long brown hair which may come from the animal about which armchair experts are so sceptical". If the author had checked this statement with the Museum he would have found that in fact no such piece of skin is known there.

In the class of over-confident assertions are the following. "I have shown that the Earth may still hold plenty of large unknown animals" (p. 81); "Herds of hundreds or even thousands of mammoths could easily live there [in the Siberian taiga] without running the least risk of being seen by man" (p. 350); of the alleged pygmy gorilla, named by Elliot *Pseudogorilla mayema* in a notoriously unreliable work, he says (p. 63) "The existence of this species, about which we know so little, has never been in doubt". Reference to current African primate literature would have shown Dr. Heuvelmans that, on the contrary, this name has long been relegated to the synonymy of the ordinary lowland gorilla, *Gorilla gorilla*. Do elephants, rhinoceroses and hippopotamuses really make the Earth shake, as we are told (p. 449) after reading of a Belgian in the Congo who felt a violent earthquake when a mysterious giant beast moved off unseen in the forest?

The basic natural history is often unreliable. It is not true that (p. 197), apart from species imported by man—the dingo and the rats, and several bats which could have flown across the sea—there are no wild placental mammals in Australia. There is a varied native rodent fauna, including 17 genera with 79 species, which has evolved over a very lengthy period in that continent. There are no macaques in Central Africa (p. 358). The account of the habits and distribution of the sea otter, *Enhydra lutris*, of the North Pacific coasts, in connexion with its suggested identification as the waitoreke of New Zealand mountain waters, is largely inaccurate. The "pack of 400 seen swimming down the Californian coast in 1938" (p. 248) (presumably on their way to the Antipodes) turns out on investigation of the source quoted to refer to a much lower number of resident sea otters recovering their former status on the Californian coast, and it would be interesting to know how the "large numbers [of sea otters] along the coast of southern Chile, which seems to imply an extraordinarily long migration" reported by a quoted observer, were identified. There is a local South American coastal otter, *Lutra felina*, found commonly in those waters, which probably accounts for this confusion.

Despite these numerous criticisms, I found this book, illustrated by many photographs and sketches, very readable and sometimes stimulating. One can part company with many of the author's conclusions without denying that he has produced a useful work. He may be reassured to learn, meanwhile, that zoologists are not all quite as blind and deaf as he suggests, and that while they are waiting for some of the animals so vividly described here to appear in the flesh, they are not entirely idle. In the five years, for example, since the publication of the original French edition of this book, no less than four distinct and quite unexpected new genera of small mammals (all small enough to be beneath Dr. Heuvelmans notice) have been described from tropical Africa, and no doubt other regions will contribute their quota in due course.

R. W. Hayman

MARKET STRATEGY AND ECONOMIC SURVIVAL

Strategy and Market Structure

Competition, Oligopoly, and the Theory of Games. By Dr. Martin Shubik. Pp. xviii+387. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 64*s*. net.

IN 1944 there appeared "Theory of Games and Economic Behavior", the pioneer work by von Neumann and Morgenstern, full of promise for a uniform and dynamic theory of the firm on a new game-theoretic basis. In the subsequent fifteen years, game theory was developed and applied in many directions, but not to any effect in the theory of oligopoly and monopolistic competition, of the firm under various market conditions. What was sown in 1944 is at last bearing fruit, ample evidence being provided by this text by Dr. Shubik. It is possible to envisage now the bridging of that wide gap, disturbing to so many economists, between the traditional theory of the firm and practical business as understood by institutional economists and business schools. This is not to say that Dr. Shubik has constructed the bridge. His book is still rather severely theoretical. But he does take into account many factors which have hitherto been rather baffling—the influence of the financial strength of a firm, its technical position and its corporate form—and he has so framed his theory that empirical work can be based upon it.

Dr. Shubik achieves two quite different objectives in dividing his book into two halves. In the first part he makes a game-theoretical exposition of existing theory and throws much new light on it. In a firm under pure competition or pure monopoly, the *entrepreneur* makes his decisions on his own and economic theory has long been able to handle the situation. Under what can be described either as oligopoly or as monopolistic competition, at least one firm in planning strategy on the market for a product must take into account the influence of at least one other firm on the same market. There is then a great variety of possible market structures, according to the varying number of firms and their different situations (for example, on transport costs), according to the extent of product differentiation, and according to the relative bargaining powers or market leadership of the firms. There is competition between firms but also co-operation, coalition and collusion. This is just the kind of situation to be exploited by game theory. Duopoly as analysed by Cournot and Edgeworth is such a special case that it cannot serve for generalization. The theory of monopolistic competition as elaborated by Chamberlin and Joan Robinson is piecemeal and lacking in the essential unifying principles. Dr. Shubik succeeds in his game-theoretical approach where the traditionalist development failed.

In the second part, Dr. Shubik attempts something even more important. This is a sketch of a truly dynamic theory of oligopoly, or rather the basis on which such a theory might well be built. He allows for imperfect information among the constituent firms on a market. His starting point is the concept of a particular type of game, the game of economic survival. A game of survival or ruin is well known as an extension of an ordinary zero-sum game, applied in successive periods until one player uses all his funds and is ruined. A game of *economic* survival is again a zero-sum game as long as the contestants survive (for example, splitting up a given market) but with a bonus to the ultimate survivor. On this view, the economic activity of the firm is to maximize something, generally the stream of dividends over time, but subject to attention being paid to survival. Not all the experts in this field go all the way with Dr. Shubik in his development of the theme, but none can deny that he is pursuing with great ingenuity something very well worth while. This is an important book, not only for game-theorists, but for all economists.

R. G. D. ALLEN

THE DARK AGE IN AFRICA

Old Africa Rediscovered

By Basil Davidson. Pp. 287+28 plates. (London: Victor Gollancz, Ltd., 1959.) 25*s*. net.

THIS book is an attempt to accomplish the well-nigh impossible; but because the author knows a great deal about the late prehistory of many parts of Africa and can put down his knowledge in a clear and interesting manner, it is well worth perusal. It has been said that a few years ago such a work would have been impossible owing to lack of information; it can equally be said that a few years hence such a work will be quite impossible, except in the form of many volumes, because of too much data. At the same time, the reader should clearly understand the scope of the book. It is true that in the short introductories there is mention of pluvials, early lines of migrations, etc., but the author is not really concerned with very early times, his interests centring on the epoch from about 500 B.C. to the opening up of the continent by the Europeans.

As this is, perhaps, the period least understood in most African regions, the book will be very much welcomed. Of necessity the areas dealt with are somewhat scattered, but the account of the pre-Portuguese civilizations, which flourished along the east coast, and which Dr. Mathew has done so much to reconstruct, is of especial interest. Much, however, has not been included. The bulk of the rock-shelter art, at least in the Union of South Africa, falls within the author's period—indeed some of the finest of the polychromes probably date no farther back than the sixteenth century A.D. *Pace* one carbon dating, it is quite likely that the same applies to the Nachikufan culture of Northern Rhodesia at least in its later phases. This is indeed mentioned but discussed as dating to 4000 B.C. But the dating of early cultures in Africa is a notoriously controversial subject and need not be pursued further here.

It is true that the spine of the Sahara desert has been well-nigh impossible for human migration since Gamblian (last pluvial) times, but subsequent wet phases, the last one of no vast antiquity, have permitted human occupation in the fringes bordering the spine both on the north and the south, and once again much of the rock-shelter art in these regions belongs to Mr. Davidson's period. The description of the ancient town of Meroe with its great slag heaps and the Kush civilization is very well set out, and to many readers the account of Bernard Fagg's splendid pottery figurines from Nigeria which up to now have not been well published will be welcome indeed. Quite rightly, Dr. Manny's work in West Africa is

noticed, and the early history of Benin is not forgotten. As has been said, the civilizations which grew up along the east coast of Africa and the (to some extent) contemporary Rhodesian buildings are treated at considerable length. The history of the early Congo is scarcely mentioned, nor is that of Angola, though the publications of the Diamond Company there have shed some light on its obscure part. The Union of South Africa, except for a brief account of Mapungubwe, is also omitted.

These gaps (geographically) are numerous, but it is difficult to see how everything could have been included in one short volume. What the author has given us is excellent reading, and he is to be congratulated on attempting what for many would have seemed impossible. It is a work which many people who care about Africa will warmly welcome.

M. C. Burkitt

IMPORTANT RAW MATERIALS

Cellulose Pulp and Allied Products

By Dr. Julius Grant. (Incorporating the third edition of "Wood Pulp".) Pp. xvi+512. (London: Leonard Hill (Books), Ltd., 1958.) 50*s*. net.

REFERENCE books of any size are now usually assembled by editors from chapters written by different authors—each an expert on his own subject. "Cellulose Pulp" is a survivor from an older tradition, and is the work of one author. This gives a uniformity in style, lacking in a collection of edited chapters, but anyone essaying a subject with as vast a literature as cellulose pulp sets himself a difficult task.

This book incorporates the third edition of "Wood Pulp", which was first published in 1938. Wood is quite the most important source of cellulose pulp (about 90 per cent) of the total and most of the book is concerned with the manufacture of wood pulp. The central chapters cover this well and should be of use to the general reader who is not 'highly technical' in accordance with the author's wishes expressed in the preface. Chapter 2 on history is well done, but the author could have made more of the history of wood pulp technology itself. Without wood pulp paper would have been so scarce that Forster's (Compulsory) Education Act of 1870, and similar measures in other countries, would have been useless. It is no good teaching children to read and write without adequate paper supplies, and the figures quoted by the author for paper consumption and literacy in Asia and Africa show that the problem is still with us.

Appropriately enough the main untapped source for pulp is in the tropical forests, but it is not easy to get sound information about them quickly as an enormous variety of trees grow in mixed stands and they all have different pulping properties. There is a serious sampling problem, and it is also very difficult to forecast the nature of the secondary growth, which is most important if pulping is to be put on a continuous basis.

In briefly relating his subject to cellulose technology in chapter 3 the author has run into some difficulties. The explanations of 'micelles' on page 37 and 'tracheids' on pages 49 and 50 are so short that they are misleading. Chapters 20, 21 and 22 on uses of cellulose also suffer from enforced brevity. The rayon and allied industries, and the plastics industry too, may be surprised at the small space given to their special problems. In most parts of the book sufficient references are given to enable the scientific reader to look up detailed information elsewhere, but the references to the uses of pulp are thin considering the variety of processes mentioned.

These, however, are not major issues and it is hoped that the book will be widely read for its manufacturing section. Too few people realize how important pulp technology is to our civilization.

F. Lyth Hudson

THE CASE FOR EXTRA-SENSORY PERCEPTION

The Sixth Sense

An Inquiry Into Extra-Sensory Perception. By Rosalind Heywood. Pp. 224. (London: Chatto and Windus, Ltd., 1959.) 21*s*. net.

IN this volume Mrs. Heywood, an active member of the Council of the Society for Psychical Research, has collected a mixed but well-arranged selection of those cases which seem to her and doubtless to many others to offer good evidence for extra-sensory perception. She is convinced that both in England and in the United States experiments have been conducted under rigid scientific conditions which have shown that it is possible for one person to become aware of another's thoughts and feelings where such information could not in any circumstances have been acquired by sensory means.

Beginning her study with a glance into the past, she briefly summarizes the early work of the Society for Psychical Research, stressing the value of its experiments and conclusions without, however, adequately dealing with the objections to its methods which were often voiced at the time. Proceeding with her analysis she discusses the work carried out on the problem of mediumistic communications, especially those associated with the names of Mrs. Piper, Mrs. Leonard and Mrs. Coombe-Tennant. The very puzzling "Cross-correspondences" are then dealt with, and the two chapters devoted to them form an excellent introduction, although the author fails to appreciate the reason why this material has never attracted the attention that it might deserve if we knew all the facts and not only some of them. The study is concluded by a short discussion of various quantitative experiments and a short summary of some of the explanations and hypotheses that have been offered.

As a popular and necessarily brief introduction to extra-sensory perception this book is to be recommended, although always with the proviso that the facts are as stated and that further knowledge of the conditions would confirm the interpretation put upon them. It is this lingering doubt about the accuracy of the records that tends to prevent so many scientific men from taking many of the statements of psychical research workers too seriously, and this hesitation is often better justified than at first appears.

In this book Mrs. Heywood has done little to allay such doubts. What she has done is to present a further stimulating and lucid study of extra-sensory perception which may prompt independent inquirers to initiate experiments on their own account, but only after an intensive study of the subject and of the pitfalls with which it abounds. E. J. Dingwall

TIZARD AND THE SCIENCE OF WAR*

By Prof. P. M. S. BLACKETT, F.R.S.
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The Air Defence Committee

IT is sad indeed that the newly founded institute for the study of war (the Institute for Strategic Studies) should so soon have to mourn the loss of one of its most distinguished members (*Nature*, **185**, 209; 1960). I wish to attempt to assess the nature of Tizard's direct personal contribution to the Allied victory in the Second World War and to trace the great influence he exerted on the general study of war, which is, of course, the explicit object of the Institute for Strategic Studies. All men's careers have much that is accidental in them, and no doubt there was an element of chance in the selection in 1934 by the Air Ministry of Sir Henry Tizard, then rector of the Imperial College of Science and Technology, as chairman of the Committee for the Scientific Survey of Air Defence. But there was none in the brilliant and decisive manner in which Tizard exploited the opportunities thus given him, at a decisive moment of world history.

The military and political outlook of the middle 1930's was indeed ominous. The world economy was just beginning to stagger uncertainly out of one of the most disastrous slumps of history; Hitler had come to power in Germany and was openly preparing for war; the advance of aeronautical science and engineering had at last gone far enough to make it possible that air power might be a vital factor in a major war—if this were so, then the extreme vulnerability of Great Britain with her high population density and her close proximity to the mainland of Europe, which only too easily might fall into enemy hands, became of major military importance. As far as there was a military doctrine of the day, it seems to have been expressed in Baldwin's famous phrase in 1933 that "the bomber will always get through".

This, then, was the background to the first meeting in January 1935 of what became universally known as the "Tizard Committee"; its official terms of reference were: "To consider how far recent advances in scientific and technical knowledge can be used to strengthen the present methods of defence against hostile aircraft". In addition to the chairman there were originally only three members: H. E. Wimperis (then director of research at the Air Ministry), A. V. Hill (physiologist at University College, London) and myself, with A. P. Rowe (later to become the director of the famous Telecommunications Research Establishment) as secretary.

During its five years of existence—the Committee was dissolved during the first years of the War—four things of major importance were achieved, mainly through Tizard's vigorous and persuasive personality. Radar, which in Great Britain originated with Robert Watson Watt at the Radio Research Laboratory of the National Physical Laboratory, was energetically supported and, with the enthusiastic support of senior airmen, introduced secretly into the Air Force. When war came in 1939 the whole east and south-east coasts of England had their radar chains operational, and this was a decisive factor in the winning of the Battle of Britain in 1940.

The second indirect but very real achievement was to bring the senior officers of the Armed Services into much closer intimacy with the research and development scientists in the Government establishments. Tizard's gifts, which will be referred to again, of inspiring confidence in young and old, in those in authority and in those at the bench or on the gun-sites: his passion for getting things done and his flair for interpreting the military requirements to the bench worker, and the technically possible to the Service chiefs: all these greatly enlivened and made more productive the scientific and technical side of the Air Force in the first instance, and then by example the other two Services.

The third achievement was the creation of mutual confidence and understanding between serving officers and university scientists; so that, when need arose, and it did arise very soon, many of the best academic research workers flocked out of the universities into radar stations, and later into Service experimental establishments, where they became a vital part of the brilliantly creative, and sometimes obstreperous, teams, whose work had so profound an effect on the waging of the War.

The fourth achievement was the recognition that scientifically trained research workers had a vital part to play, not only (as of course was traditional) in the development of the weapons and gadgets of war but also in the actual study of operations. It was due to Tizard's personal initiative as early as 1936, before, in fact, radar data on approaching aircraft were available, that civilian scientists were attached to the Fighter Station at Biggin Hill to study the art of controlled interception, as it would have to be done, when a year or two later the radar chain would become operational. This experiment seems to have been the first official recognition that the actual operations of modern war are so complicated and change so fast that the traditional training of the serving officers and personnel is inadequate. In fact, many of the operational problems which arise when new equipment comes into service require for their solution the aptitudes of the scientific research worker: for he is trained to apply scientific methods to elucidate hitherto unknown and complex phenomena.

The Biggin Hill experiment was the first step towards the fully fledged Operational Research Sections attached eventually to all the major Commands of all three Services. A further important step was taken when, at the outbreak of the War, A. P. Rowe, then in charge of radar research at Bawdsey,

* Tizard Memorial Lecture delivered on February 11 before the Institute for Strategic Studies.

sent a small group of scientists to Headquarters Fighter Command to study the actual use of the radar data so as to keep the radar scientists in the establishments briefed both on the actual performance of the sets in operations and on the real operational needs of Fighter Command. Out of this group grew the Fighter Command Operational Research Section as an integral part of the Command organization. Analogous developments took place at Anti-Aircraft, Coastal and Bomber Commands, and also at the Admiralty and the War Office. These developments implied a great measure of mutual trust and understanding between the senior Service officers and the often brash and initially very ignorant scientists—ignorant, that is, of most things that went on outside a university research department. This intimate relationship between professional fighting men in charge of operations and research scientists proved highly profitable, and some of the later operations of the War were carried out under a close scientific and statistical scrutiny. The pattern of organization and method worked out in Britain during the first years of the War stimulated similar developments in the United States. Hitler's Third Reich saw no such collaboration. No doubt the almost unbroken German military successes of the first war years confirmed the highly competent military staffs in the view that they had no need to seek help from outside scientists, however brilliant. When the tide of war swept against Germany it was too late. Luckily for the Allies, Germany never produced its Tizard.

The importance of bringing scientists into close touch with operations had been clearly foreseen during the early days of the Tizard Committee. It was well expressed by the Secretary of State for Air, Lord Swinton, then Sir Philip Cunliffe Lister. In his book "I Remember" he wrote: "There was nothing new in the use of men of science as advisors to Service Departments and to the Committee of Imperial Defence. . . . There was nothing new, therefore, in calling in science to help; but from the moment I went to the Air Ministry, I felt that in the air, with all its unknown possibilities, we wanted a much closer and more intimate relationship. The scientists who were to work with us must be from the start an integral part of the Air Staff". Of the Tizard Committee he wrote: " . . . they were at the heart of operational planning. That relationship was, and I believe always will be, the key to success in scientific co-operation".

Prominent among the senior Air Force officers who welcomed and co-operated closely with the Tizard Committee were Air Marshal Sir Cyril Newall, chief of the Air Staff 1937–40; Air Marshal Sir Hugh Dowding, Air Member for Research and Development 1930–36 and then commander-in-chief Fighter Command, and so the first operational user of radar; Air Marshal Sir Wilfred Freeman, who succeeded Dowding in 1936 as member for Research and Development; Air Marshal Tedder (now Lord Tedder), who was director-general for Research and Development during 1938–40; and Air Marshal Sir Philip Joubert, then air officer commanding Fighting Area and later commander-in-chief Coastal Command.

For the encouragement of the later developments of the application of scientific method to tactics and strategy, much credit goes to General Sir Frederick Pile at Anti-Aircraft Command, Admiral Sir George Creasy, then director of Anti-Submarine Warfare at the Admiralty, and Air Marshal Sir John Slessor, now a vice-president of the Institute for Strategic Studies. During Slessor's time as commander-in-chief Coastal Command 1943–45, the scientific analysis of the anti-submarine war became an accepted part of operational control, and statistics of air hours flown, rates of search, U-boat sightings, etc., were discussed at the fortnightly U-boat meetings at No. 10, Downing Street—once goading a senior officer to protest that wars were won by weapons and not by slide rules, so giving rise to the half-derisive but also appreciative term 'slide rule strategy'.

Unfortunately, Tizard published little about his own contribution to these developments, though no doubt much of great interest will be found in Whitehall files, when these become available to historians. So far as I know, his lecture to the Royal United Service Institution in 1946, entitled "Science and the Services", and his Haldane Memorial Lecture at Birkbeck College in 1955, entitled "A Scientist In and Out of the Civil Service", are almost all that are available: and in these, with characteristic modesty, there is little about his own achievements. So the history of these vital years has to be pieced together from personal memories.

Controversy on Priorities

Although the Air Defence Committee started up in January 1935 with only Tizard, Hill, Wimperis and myself as members, in July of the same year the Secretary of State for Air, under pressure from Mr. Winston Churchill, enlarged it by the addition of Prof. F. A. Lindemann (afterwards Lord Cherwell). It was not long before the meetings became long and controversial: the main points of dispute concerned the priorities for research and development which should be assigned to the various projects which were being fathered by the Committee. For example, Lindemann wanted higher priority for the detection of aircraft by infra-red radiation and for the dropping of parachute-carrying bombs in front of enemy night bombers, and lower priority for radar, than the other members thought proper. On one occasion Lindemann became so fierce with Tizard that the secretaries had to be sent out of the committee room so as to keep the squabble as private as possible. In August 1936, soon after this meeting, A. V. Hill and I decided that the Committee could not function satisfactorily under such conditions; so we resigned. A few weeks later Lord Swinton re-appointed the original Committee without Lindemann, but with Prof. E. V. Appleton, and a few months later with Prof. T. R. Merton, as additional members.

During the years 1935–39 the Committee investigated and assessed a large number of projects, good and bad; we visited many establishments and discussed all aspects of air defence problems with air marshals, with pilot officers and with scientists in their laboratories. Pre-eminent, of course, was radar, both ground and air-borne. The story of radar has often been told, and all I wish to emphasize is the vital part played by the confidence which Tizard did so much to build up between the senior airmen, the research workers and the responsible Cabinet ministers. The full backing of the Committee became an effective way of getting high priority given to a project. Without such mutual trust the scientific development of radar could not have forged ahead at such speed, nor would the tens of millions of pounds have been provided so rapidly and secretly by the Treasury to build the radar chain.

In 1937, Tizard was made chairman of another Committee, this time for the Scientific Survey of Air Offence, modelled on the lines of the Air Defence Committee. However, it failed to come to grips with the problems of the use of bombers and made no decisive impact. Possibly Tizard himself was too heavily involved in air defence problems easily to switch his interest: possibly the failure was partly due to the not very sympathetic response by those in the Air Force then concerned with bombing policy. Whatever the cause, I think it would be fair to hold that until the War was well advanced, Bomber Command was less scientifically minded than either Fighter, Coastal or Anti-Aircraft Command. Moreover, it was just this failure to think and analyse scientifically which delayed for too long the recognition of the extreme ineffectiveness of our early bombing offensive. Sir Winston Churchill tells how Prof. Lindemann in 1941 informed him that, of the bomber crews who thought they had found their target, it had been shown by analysing flash photographs that two-thirds were not within five miles of it. Once these facts were fully recognized, high priority was put on navigational aids of various kinds, and matters greatly improved.

Tizard's Mission to the United States

In November 1939, Tizard, then scientific adviser to the Air Ministry, suggested that A. V. Hill should go to Washington to act as scientific adviser to work with the Air Attaché, so as to get scientific help on defence problems from the United States. Since scientific developments were likely to play a major part in determining the issue of the War, the importance of attempting to get official approval for Britain to tap the vast scientific resources of the United States was very great. In a typically apt phrase Tizard described his policy as that of "bringing American scientists into the War before their Government". In this he succeeded in a startlingly successful manner. When A. V. Hill reached Washington, he realized that this objective could only be attained if a complete interchange of scientific secrets between the two countries could be arranged. With the help of the British Ambassador, Lord Lothian, President Roosevelt became convinced of the importance of such interchange, as were also the American Service chiefs. On the other hand, the task of convincing the British Government that it was useful and safe to entrust our most closely guarded secrets to the scientists and Armed Services of a then neutral country, with not a few anti-British elements in it, was not easy. But at last Tizard succeeded; and in September 1940, as the 'Blitz' on London started, the Tizard Mission, with Cockcroft as second in command, set out for the United States with the famous black box containing samples, blueprints and reports on nearly all important new British war devices. These included radar, fire control, under-water detection, aircraft turrets, Whittle's jet engine and, above all, a sample 9·1 cm. resonant cavity magnetron.

Within a few days American scientists had the magnetron working, to find it gave a thousand times the power formerly available. This remarkable invention, due to Randall and Boot working in Oliphant's laboratory at the University of Birmingham, was called by Watson Watt "a radically new and immensely powerful device which remains the heart of every modern radar equipment".

An assessment of the importance of this interchange of secret military information is given in James Phinney Baxter's book "Scientists Against Time", published in 1946 with a foreword by Vannevar Bush. The writer comments on the contents of the black box as "the most valuable cargo ever brought to our shores" and "the single most important element in reverse lease lend". He goes on: "In the early days of scientific interchange, the British gave more than they received". Later the debt was repaid many-fold; in particular, for example, by the superb centimetric anti-aircraft radar set *SCR*-584 and the proximity fuse which together led to such brilliant successes by Anti-Aircraft Command during the *V*.1 attack on England in 1944. The former set was a product of the famous Radiation Laboratory at the Massachusetts Institute of Technology, which was, in fact, founded as a result of a meeting of the Tizard Mission with American scientists in September 1940.

This imaginative act of trust, which Tizard and A. V. Hill first envisaged and finally forced through Whitehall, had immensely beneficial effects on the scientific aspects of the Allied war effort. Cockcroft reminds us that the Mission was magnificently organized by Tizard and that he had the inspiration to bring a mixed team of serving officers and scientists. For the first time our American friends heard civilian scientists discussing authoritatively the instruments of war and then heard the Service people following on with practical experience.

The Bombing Controversy

When Tizard returned to England in September 1940 on the completion of his American Mission, he worked full time at the Ministry of Aircraft Production and later became a member of the Air Council. The Air Defence Committee itself died a natural death, since an advisory committee of part-time members was not useful under fully mobilized war conditions.

There is little doubt that Tizard found less than satisfaction in what he could achieve during the years 1941 and 1942. In spite of his wide knowledge of the Government machine, he seems to have been genuinely disappointed to find that the Air Council rarely discussed strategy or tactics, but often minor administrative matters: he related that at one meeting of the Air Council the main business was the inspection of an exhibition of different designs of W.A.A.F. underwear! A deeper difficulty underlay the frustrations of this period. For it was not at all easy for a Service Ministry to incorporate a man as senior as Tizard in its taut, executive, war-time machine. On one hand he was too senior to do his own devilling among the files at the lower levels of the machine, where much of the real work of a department is done —to be sure, he was too impatient to have been very good at this—and on the other hand, he had too much self-respect to acquire influence by becoming a courtier. Tizard evidently felt that he was not wanted in Whitehall and so he accepted the presidency of Magdalen College, Oxford, where he remained, apart from some Government advisory work and several important visits abroad, until after the end of the War.

Shortly before he went to Oxford, Tizard became involved in a controversy about bombing policy which had far-reaching consequences. The interest of this story is not only its importance at the time,

but also that it concerned problems of the theory and practice of bombing which, now with the advent of nuclear weapons, is giving rise to so much deep thought, subtle analysis and high controversy, carried on not least by members of the Institute for Strategic Studies.

As I remember it, what happened was this. In the early spring of 1942 the Prime Minister was extremely anxious that everything possible should be done by Bomber Command to help the hard-pressed Soviet armies at Stalingrad. About April a Cabinet paper (which was known to have been written in Lord Cherwell's office, Cherwell then being Paymaster-General and the Prime Minister's scientific adviser) was issued on the probable effect on Germany of the British bombing offensive over the next eighteen months, that is, until the early autumn of 1943. The paper laid down the policy of directing the bombing offensive primarily against the German working-class housing—middle-class housing was too spread out to be a good target and factories or military installations were too difficult to find and hit. So far as my memory goes, the paper claimed that it should be possible within the stated period to destroy 50 per cent of all houses in all towns of more than 50,000 population in Germany, if Britain concentrated all her efforts on the production of bombers and used them for this purpose. Tizard studied this paper in detail and concluded that the estimate of the number of houses likely to be destroyed in the next eighteen months was five times too high. At that time I was director of Operational Research at the Admiralty, and I was also asked to comment on the Cabinet paper. I came independently essentially to the same conclusion as Tizard—I think I estimated the error as six-fold. The main mistake made in the Cabinet paper was to assume that all bombers which would be delivered from the factories in the next eighteen months would in the same period have dropped all their bombs on Germany. The year or more from the completion of a bomber to the completion of its average operational life of twenty sorties had not been fully allowed for. The bombing survey after the War showed that the number of houses actually destroyed in the assigned period was only one-tenth of the estimate in the Cabinet paper: this agreed rather closely with Tizard's and my estimates, allowing for reduced numbers of bombers actually supplied to Bomber Command.

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The story goes that at that time in the Air Ministry it was said of anyone who added two and two together to make four, "He is not to be trusted; he has been talking to Tizard and Blackett". Less agreeable stories circulated: that anyone who made such calculations must be a defeatist.

So far as I know, Tizard never wrote anything of this period and spoke but sparingly; but he did comment after the War in his Royal United Service Institution lecture on the theory and practice of aerial bombing. "Experience has shown that a nation, with toughness, stamina and a will to live and work, can stand far more punishment in the form of bombardment of cities and homes than most people thought possible before the trial. No one thinks now that it would have been possible to defeat Germany by bombing alone. The actual effort in man-power and resources that was expended in bombing Germany was greater than the value in man-power of the damage caused. . . ."

If only Tizard's Offence Committee had succeeded as well as his Defence Committee, and if as a result a strong and trusted operational research section had been earlier established at Bomber Command, I think it likely that the numerical error on which the area bombing campaign was initiated would not have been made and that great advantage to the war effort would have resulted.

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Too much, however, must not be made of personal factors, strong as these no doubt were. For the entry of academic scientists into fields of military strategy and tactics brings to them responsibilities for advising on courses of action on which the survival of one's country—or, to-day, in an age of nuclear weapons, the survival of civilization—may depend. So real differences of technical judgment become allied to differences of temperament and lead to lasting and often bitter struggles. The war-time controversy on bombing policy with conventional explosives and the strains that it engendered in Whitehall were but a minor foretaste of the still more bitter controversy, particularly in the United States, engendered by atomic weapons, which has left such a trail of personal antagonisms behind it.

Tizard in Whitehall, 1946–52

The result of the 1945 General Election with the consequent return of Cherwell to Oxford opened the way for Tizard to return to Whitehall. The opportunity soon presented itself, for in 1946 the new Government, headed by our President, Lord Attlee, invited him to become chairman of two new high-level advisory bodies, the Defence Research Policy Committee and the Advisory Council on Scientific Policy. Widespread acclaim from both scientists and military greeted both the formation of these bodies and the choice of Tizard to head them. Thus Tizard, for the third and last time, became a full-time Civil servant. What he achieved in the six years to his retirement in 1952 was by no means the least of his service to the nation, for there is no doubt that a great part of the credit for the growth to maturity of these two committees must go to him.

These last six years of his active life were in a real sense the fulfilment of his quarter-of-a-century-old belief in the importance to the life and prosperity of Great Britain of a close relationship between the administrative and scientific worlds, a belief which was one of the factors which led him in 1920 to forsake an academic for a Civil Service career.

It would probably be generally agreed that the Defence Research Policy Committee has proved a more effective instrument than the Advisory Council on Scientific Policy. This is certainly mainly due to the much narrower and administratively well-defined field of its activity. The executive authority residing in the Defence Ministry and the Chiefs of Staff, together with the related financial

power of the Supply Ministries, gives them almost complete control of all defence matters, whether in the Government or in industry. So any advice from the Defence Research Policy Committee, on which these Government bodies are represented, is likely to be relevant and acted upon. Some of those who were intimately concerned with the working of the Committee hold that Tizard moulded it into the finest organization for co-ordinating the scientific and technical aspects of war policy which has ever existed in the Western world. During his time as chairman, from 1946 until 1952, Tizard's influence was exercised over a wide range of important and complex problems, including those arising from atomic weapons, super-sonic fighters, *V*-bombers and guided missiles. He shared much of the responsibility for developing the rocket range at Woomera, and when some Australians were worried that it would cost them a lot of money and perhaps no one would use it, he told them not to worry as it would attract work and men as a magnet attracts iron filings. A good example of his flair for the important practical action arose out of a discussion concerning the rocket and guided missile programme. After years of intricate debate on what the research and development programme should be, when a practical plan was eventually hammered out, Tizard ruled that the implementation of the programme should be carefully watched but that there should be no alteration or even discussion of the basic programme for two years. This ruling is reminiscent of his advice to fishermen: "Don't waste time changing flies and always know what the fishes are doing".

In his Haldane Memorial Lecture at Birkbeck College in 1955, Tizard referred to the creation of the Defence Policy Research Committee thus: "It was a revolution in organization. It was not until a short time before the Second World War started that scientists began to exercise an influence on the tactical use of weapons and later on strategy. I believe I can truthfully say that the very idea that they should interfere in such matters was repugnant to senior officers twenty years ago. It was the experience of war that caused the revolution and the appointment of the new Committee, far from being resisted by the Chiefs of Staff, was, in fact, initiated by them". "The Chairman . . . has full access to all relevant information, and attends the meetings of the Chiefs of Staff Committees not only when he is specially asked to do so but whenever he judges that his presence will be useful. No major recommendation on Defence Policy is made by the Chiefs of Staff without his knowledge and assistance".

The role of the Advisory Council on Scientific Policy, on the other hand, was much more difficult to define. Its advisory activities ranged over a wide field, including scientific and technological education and man-power, the organization of Government science and indirectly the efficiency of research and development in private industry. Taking into account the rigid autonomy of Government Departments and the privacy of private enterprise, the Advisory Council, with neither money nor executive authority, had from the beginning, and has still, a difficult task. Perhaps its main success has been in the field of scientific and technical man-power. Its relative lack of impact on the national life is not to be attributed to lack of ability of Tizard, but to the essential difficulty of injecting a small degree of direction into a social system where outside a few fields such as education and defence the word planning is not seldom used as a term of ridicule. These difficulties are currently receiving much public attention, stimulated by the vast scientific and technological power of the United States, by the much faster industrial growth of some of our European competitors and by the massive technological and economic advance of the highly directed economy of the Soviet countries. Here are problems galore for Tizard's successors on the Advisory Council on Scientific Policy to solve.

The changes that have taken place in the world of military science in the past twenty-five years, that is, since Tizard's Air Defence Committee was formed, have indeed been immense. The revolution in weapons is, of course, the most obvious, and the ultimate origin of the other organizational and psychological changes. In the 1930's it was certainly important in Great Britain to bring into the defence field from academic life as many highly trained and critical minds as possible; now in 1960 the outburst of analytical thinking and writing on military subjects by scientists, lawyers, historians, psychologists and philosophers, and, of course, by retired generals, is becoming almost overwhelming and is one of the fundamental causes of the foundation of the Institute for Strategic Studies. No serving officer could possibly find the time to read all the current literature; those amateurs like myself who write on these matters have no time to read all that the other amateurs write. The flood is greatest in the United States, where many universities have their military or foreign policy research groups or institutes, where brilliant academic minds analyse and dissect our present Western military discontents and are by no means shy of ingenious solutions. Traditionally, Britain has been averse to thinking about war in between fighting wars; once they are over we tend to forget about them until the next time. Even to-day there are only two academic posts in our universities concerned with military studies. The Institute for Strategic Studies is attempting, and I think successfully, partially to fill this gap.

This huge expansion of thinking about military subjects is, of course, largely due to nuclear weapons, and is to be welcomed as a sensible response to a highly complex and dangerous situation. On the other hand, I cannot convince myself that the practical content of the response has everywhere proved adequate to the challenge. The intellectual level of much of this discussion is of the highest, so high that I find much of it very hard to understand, and I wonder sometimes whether it is all rooted in military and political reality. The policies recommended by many of these studies seem not always to have been felt through to actual potential action. I think the essential prerequisite of sound military advice is that the giver must convince himself that if he were responsible for action, he would himself act so. During the War, when the Operational Research Group at the Admiralty had proved intellectually to themselves that big convoys were safer than small ones, before we advised the Navy to make this major change, we had to decide whether we really believed in our own analysis. I personally convinced myself that I did, by the conviction that if I were to send my children across the Atlantic during the height of the U-boat attacks I would have sent them by a big rather than by a small convoy. I think that occasionally analogous personal tests of belief in one's own recipes may be useful in the study of nuclear war. Perhaps part of the fault has

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These last six years of his active life were in a real sense the fulfilment of his quarter-of-a-century-old belief in the importance to the life and prosperity of Great Britain of a close relationship between the administrative and scientific worlds, a belief which was one of the factors which led him in 1920 to forsake an academic for a Civil Service career.

It would probably be generally agreed that the Defence Research Policy Committee has proved a more effective instrument than the Advisory Council on Scientific Policy. This is certainly mainly due to the much narrower and administratively well-defined field of its activity. The executive authority residing in the Defence Ministry and the Chiefs of Staff, together with the related financial

power of the Supply Ministries, gives them almost complete control of all defence matters, whether in the Government or in industry. So any advice from the Defence Research Policy Committee, on which these Government bodies are represented, is likely to be relevant and acted upon. Some of those who were intimately concerned with the working of the Committee hold that Tizard moulded it into the finest organization for co-ordinating the scientific and technical aspects of war policy which has ever existed in the Western world. During his time as chairman, from 1946 until 1952, Tizard's influence was exercised over a wide range of important and complex problems, including those arising from atomic weapons, super-sonic fighters, *V*-bombers and guided missiles. He shared much of the responsibility for developing the rocket range at Woomera, and when some Australians were worried that it would cost them a lot of money and perhaps no one would use it, he told them not to worry as it would attract work and men as a magnet attracts iron filings. A good example of his flair for the important practical action arose out of a discussion concerning the rocket and guided missile programme. After years of intricate debate on what the research and development programme should be, when a practical plan was eventually hammered out, Tizard ruled that the implementation of the programme should be carefully watched but that there should be no alteration or even discussion of the basic programme for two years. This ruling is reminiscent of his advice to fishermen: "Don't waste time changing flies and always know what the fishes are doing".

In his Haldane Memorial Lecture at Birkbeck College in 1955, Tizard referred to the creation of the Defence Policy Research Committee thus: "It was a revolution in organization. It was not until a short time before the Second World War started that scientists began to exercise an influence on the tactical use of weapons and later on strategy. I believe I can truthfully say that the very idea that they should interfere in such matters was repugnant to senior officers twenty years ago. It was the experience of war that caused the revolution and the appointment of the new Committee, far from being resisted by the Chiefs of Staff, was, in fact, initiated by them". "The Chairman . . . has full access to all relevant information, and attends the meetings of the Chiefs of Staff Committees not only when he is specially asked to do so but whenever he judges that his presence will be useful. No major recommendation on Defence Policy is made by the Chiefs of Staff without his knowledge and assistance".

The role of the Advisory Council on Scientific Policy, on the other hand, was much more difficult to define. Its advisory activities ranged over a wide field, including scientific and technological education and man-power, the organization of Government science and indirectly the efficiency of research and development in private industry. Taking into account the rigid autonomy of Government Departments and the privacy of private enterprise, the Advisory Council, with neither money nor executive authority, had from the beginning, and has still, a difficult task. Perhaps its main success has been in the field of scientific and technical man-power. Its relative lack of impact on the national life is not to be attributed to lack of ability of Tizard, but to the essential difficulty of injecting a small degree of direction into a social system where outside a few fields such as education and defence the word planning is not seldom used as a term of ridicule. These difficulties are currently receiving much public attention, stimulated by the vast scientific and technological power of the United States, by the much faster industrial growth of some of our European competitors and by the massive technological and economic advance of the highly directed economy of the Soviet countries. Here are problems galore for Tizard's successors on the Advisory Council on Scientific Policy to solve.

The changes that have taken place in the world of military science in the past twenty-five years, that is, since Tizard's Air Defence Committee was formed, have indeed been immense. The revolution in weapons is, of course, the most obvious, and the ultimate origin of the other organizational and psychological changes. In the 1930's it was certainly important in Great Britain to bring into the defence field from academic life as many highly trained and critical minds as possible; now in 1960 the outburst of analytical thinking and writing on military subjects by scientists, lawyers, historians, psychologists and philosophers, and, of course, by retired generals, is becoming almost overwhelming and is one of the fundamental causes of the foundation of the Institute for Strategic Studies. No serving officer could possibly find the time to read all the current literature; those amateurs like myself who write on these matters have no time to read all that the other amateurs write. The flood is greatest in the United States, where many universities have their military or foreign policy research groups or institutes, where brilliant academic minds analyse and dissect our present Western military discontents and are by no means shy of ingenious solutions. Traditionally, Britain has been averse to thinking about war in between fighting wars; once they are over we tend to forget about them until the next time. Even to-day there are only two academic posts in our universities concerned with military studies. The Institute for Strategic Studies is attempting, and I think successfully, partially to fill this gap.

This huge expansion of thinking about military subjects is, of course, largely due to nuclear weapons, and is to be welcomed as a sensible response to a highly complex and dangerous situation. On the other hand, I cannot convince myself that the practical content of the response has everywhere proved adequate to the challenge. The intellectual level of much of this discussion is of the highest, so high that I find much of it very hard to understand, and I wonder sometimes whether it is all rooted in military and political reality. The policies recommended by many of these studies seem not always to have been felt through to actual potential action. I think the essential prerequisite of sound military advice is that the giver must convince himself that if he were responsible for action, he would himself act so. During the War, when the Operational Research Group at the Admiralty had proved intellectually to themselves that big convoys were safer than small ones, before we advised the Navy to make this major change, we had to decide whether we really believed in our own analysis. I personally convinced myself that I did, by the conviction that if I were to send my children across the Atlantic during the height of the U-boat attacks I would have sent them by a big rather than by a small convoy. I think that occasionally analogous personal tests of belief in one's own recipes may be useful in the study of nuclear war. Perhaps part of the fault has

been the relative silence of some professional fighting men, often may be for good Service reasons.

What I feel is needed now is more effective analytic activity within the Armed Services by those senior officers who would have, in the event of war, the actual responsibility of waging it—and conceivably of pressing nuclear triggers. All generals will agree that atomic war is much too serious a matter to be left to professors.

Tizard the Man

History has shown that Wimperis's choice in 1934 of Tizard as chairman of the famous Air Defence Committee was a very wise one: he had just the right mixture of academic and military background to enable him to build a bridge between these two very distinct worlds. Son of a captain in the Navy, who had been navigator to the *Challenger* Expedition and later Assistant Hydrographer, and who was also a Fellow of the Royal Society, the young Tizard had also been destined for the Navy but had been rejected because of eyesight. This, however, did not prevent him from becoming a pilot in the Royal Flying Corps during the First World War, where he rose to the rank of lieutenant-colonel and was awarded the Air Force Cross. Previously to this he had taken both mathematics and chemistry at Oxford, had spent a year in Berlin with Nernst, and had made researches in both physical chemistry and physics. Early in 1917 he was put in charge of all experimental flying at Martlesham Heath and later in the year went to headquarters of the Ministry of Munitions. In his lecture to the Royal United Service Institution in 1946, Tizard, commenting on his experiences at Upavon as a young Royal Flying Corps pilot, wrote of the "absence of any sign of interest on the part of the War Office . . . in the scientific development of the new fighting arm . . . as for air fighting, the enemy called the tune and we danced to it". Thus his early experiences in the Royal Flying Corps must have fixed as firmly in his mind the danger to the nation of technological inferiority in war as my experience in the Navy did in mine.

There is a well-known story of his Martlesham time. Just as he was about to take off to test a new Sopwith Camel fighter, a formation of Gotha bombers approached London. Tizard had the untested guns loaded and intercepted the bombers on their return, only to find that his guns jammed immediately. So he joined the formation and made notes about the speed and performance of the Gothas, which information was badly needed. He then waved good-bye. His most important contribution during the First World War was the development of a hitherto non-existent system of performance testing, so that afterwards no new design went into production without proper testing.

After the War he returned to Oxford and to physical chemistry, but only for a short time, as in 1920 he became assistant secretary of the Department of Scientific and Industrial Research, and secretary in 1926. Then in 1929 he became rector of the Imperial College of Science and Technology, where he remained until 1942. In 1933, he was appointed chairman of the Aeronautical Research Committee and held the post for ten years. He was thus very knowledgeable about all aeronautical matters.

With such experience and such interests it is not surprising that Tizard exploited so brilliantly the opportunity which Wimperis afforded him by making him chairman of that "grand body", as Rowe once called it. Tizard knew the Government machine well enough to realize that all committees could not achieve such great things, and in his Haldane Lecture one finds a good example of that dry wit which all his friends remember as one of his most endearing characteristics and which we all mourn to have lost. In writing of the early thirties and of the gradual growth of a machinery for scientific advice to the Government, Tizard refers to one committee in the following words: "The Committee on tsetse fly control was still sitting in 1938 without any noticeable effect on the expectation of life of these pests".

Tizard's outstanding achievement in bringing civilian scientists and serving officers into such fruitful contact was based on personal qualities of a high order. Though fundamentally of a conservative temperament and a great admirer of the virtues of tradition in national life, he was a radical in technological matters, and approached new problems and possibilities with courage, enthusiasm and originality. It is true that he often followed up too many ideas at the same time and sometimes bewildered his colleagues by alteration of course induced by the almost gad-fly quickness of mind. One reason for this was his instant readiness to look at anyone's bright ideas and to see whether they could be made to work. It is these qualities which perhaps led to him being considered a poor administrator by some people—it is true that he would sometimes chase some hare for a few weeks and then switch his interest suddenly to some more profitable quarry. However, these intellectual escapades were seldom fruitless and were an essential part of his method of spot-lighting the really important problems. When he himself had sifted the ideas thrown up by his own exuberant technical and administrative imagination he was exceedingly practical.

Throughout our long friendship, which began in 1934 when I became a member of the Aeronautical Research Committee of which he was then chairman—and what an admirable chairman he was— I have never failed to find stimulus, entertainment and enlightenment from his company. Though sometimes a little irritable, he was essentially warm-hearted and very wise. Of the individuals who have influenced me by their personality, I think Tizard comes next to Rutherford. As has already been emphasized, he had a most unusual ability to establish immediate and mutually stimulating contact with anyone doing a job; he seemed as genuinely interested in talking to a college porter, an aircraftsman or a young scientist as to a crack pilot, a professor or an air marshal. A visit with Tizard to an experimental establishment was an exhilarating experience: he left behind him a newer awareness of the tasks and a new keenness to get results.

Along with the great encouragement he so often gave to the younger men in the Services and in academic life, and his gift for inspiring them with his own enthusiasm for getting things done, his flair for setting the cat among the official pigeons, and for ignoring the normal channels, made him not always popular with the upper hierarchy. His sharp incisive wit was often exercised at the expense of the complacent, the pompous and the incompetent—among these he undoubtedly made enemies—but I have never heard of it being used at the expense of the young, the vigorous or the keen. There is a story which shows his ready wit, dating from the meeting of the British Association in Canada before the War. Tizard and a colleague inadvertently crossed over

into the United States near Niagara. When challenged by a policeman, and not having their passports with them, they produced their Association membership cards. When the policeman told them that "The American Government doesn't recognize British Science", the lightning reply came from Tizard, "Oh, that's all right, neither does the British Government". As Linstead, the rector of Imperial College, recently wrote, Tizard himself played a major part in making the crack less applicable to-day.

In his Haldane Lecture, Tizard pays the highest tribute to Lord Haldane for his achievements over four decades ago, in making official circles in Britain first conscious of the national implications of the scientific-technological revolution. In a sense Haldane was the first British Minister for Science: Tizard was his disciple and we to-day are his. I will conclude by quoting to you, as expressing most aptly what many of his intimate colleagues feel about Tizard, what he felt about Bertram Hopkinson, professor of engineering at Cambridge, and one of the outstanding pioneers in Great Britain of the application of science to war, and whose death in an aeroplane accident in 1918 was a real calamity. Tizard wrote: "He had not only the position, but the qualities, to command respect from soldiers and scientists alike: and those who served under him, and who have been able to do anything for the Services in later years, have owed our own capacity largely to his leadership and inspiration".

EXPERIMENTS TO TEST EINSTEIN'S PRINCIPLE OF EQUIVALENCE

ON January 30, the last day of the annual meeting of the American Physical Society in New York, three papers describing experiments designed to test Einstein's principle of equivalence were read. The principle announced by Einstein in 1911 states that a system in a uniform gravitational field is completely equivalent to a uniformly accelerated system, both for mechanical and for all other processes. This was suggested by the experimental result, which was first obtained by Galileo and more accurately by Eötvös in 1891, that gravitational and inertial masses are the same. From this principle, Einstein concluded that the rate of clocks at points of low gravitational potential is slower than at points of high gravitational potential, and deduced that spectral lines emitted from the Sun should show a shift to the red when compared with those produced on the Earth. He further deduced that the velocity of light is not constant in a gravitational field, so that light rays become curved, and that in all cases, in addition to an inertial mass, a gravitational mass $m = E/c^2$ is to be ascribed to energy.

If light travels in a system which has a uniform acceleration f, then the apparent Doppler velocity after a distance h is fh/c. Using the principle of equivalence, this implies that in the Earth's gravitational field the change of frequency of light is given by $\delta\nu/\nu = gh/c^2$. Alternatively, we may consider a description in which the rate of clocks changes in the gravitational field. Then, for the frequencies of the photon at the two points, we have $\nu_1 \approx \nu_0 (1 - \varphi_1/c^2)$ and $\nu_2 \approx \nu_0 (1 - \varphi_2/c^2)$ and $\delta\nu/\nu = \delta\varphi/c^2$, where φ is the gravitational potential, and $\delta\varphi = gh$.

It is interesting to consider the effects in a rotating system. Let us consider a system which rotates with angular velocity ω. Then a clock which is at rest in this system will be slowed down by an amount which increases as the clock is moved away from the axis of rotation. The time dilatation is given by:

$$t = \frac{\tau}{\left(1 - \frac{\omega^2 r^2}{c^2}\right)^{1/2}} \approx \tau\left(1 + \frac{1}{2}\frac{\omega^2 r^2}{c^2}\right)$$

where r is the distance from the axis.

An observer rotating with the system will not regard this as a special relativistic effect, since the clock is at rest in his frame; but at the clock he will observe a field of force, which he can describe by a potential, equivalent to a gravitational potential. Writing $\varphi = -\frac{1}{2}\omega^2 r^2$, we again obtain the expression $\delta\nu/\nu = \delta\varphi/c^2$.

It will be noticed that both these effects are second order in $1/c$, and are therefore extremely small. The expected shift in frequency of light from the Sun is about two parts in 10^6, and in the Earth's gravitational field, the shift amounts to 10^{-13} per cm. In a rotating system the shift can be made larger, but still not much greater than 10^{-12}, this limit corresponding to the maximum strength of materials. Repeated attempts have been made to observe the red shift in light from the Sun or from stars, but there are too many interfering processes of which little is known for convincing results to be obtained.

The situation has been completely changed by a discovery made in 1958 by R. L. Mössbauer, relating to the emission of γ-rays by nuclei in solids. It is well known that the natural width of many nuclear γ-rays, given by $\hbar/\tau$, where τ is the life-time, is extremely narrow. For example, a half-life of 10^{-7} sec. corresponds to a width of 10^{-8} volt. In a line of 10 keV., this gives a relative width of about 10^{-12}. But, in general, it is not possible to utilize this extreme resolution, because when the γ-ray is emitted, the nucleus recoils and the resulting γ-ray energy is shifted by the Doppler effect. If the nucleus were perfectly free, this would shift the line by a fixed amount; but in a substance at a finite temperature there is a large number of possible recoil velocities, resulting in a widening of the line.

Mössbauer showed that in the case of γ-ray emission from nuclei in a crystal, some γ-rays are emitted in which the recoil is taken up by the whole crystal, and therefore with a line-width which is determined by the life-time of the nuclear state. The fraction of γ-rays so emitted is given by the Debye-Waller factor, and depends particularly on the energy of the γ-ray, the elastic constants of the crystal and the temperature. It follows that when such γ-rays fall on a crystal containing the same nuclei as the emitting nuclei, there is a high probability that they will be absorbed by a resonance process. Mössbauer's original work was done with γ-rays from iridium. In

this case, at 80° K., 1 per cent of the γ-rays emitted are 'recoilless'. Mössbauer showed that a relative velocity of a few cm./sec. between the source and absorber introduced sufficient Doppler shift to destroy the resonance. In September 1959, it was shown independently at Harvard and Harwell that the nucleus iron-57 emitted γ-rays showing the Mössbauer effect to a much more favourable extent. The Debye–Waller factor is in this case 63 per cent even at room temperature, and the line-width corresponds to a Doppler velocity of 0·01 cm./sec. It was realized both at Harvard and Harwell that this was a particularly suitable nucleus to use in experiments on the red shift.

The first of the three papers before the American Physical Society was read by Prof. Pound of Harvard University, and described work by himself and Mr. Rebka. He first gave an introduction to the gravitational effects similar to that given above, and showed how the effect discovered by R. L. Mössbauer offered the opportunity of testing the gravitational shift. The apparatus consists of a 400-mc. source of iron-57 mounted on a piezo-electric transducer, which is itself mounted on a hydraulic device which can provide velocities of the order of 0·0001 cm./sec. for calibrating purposes. Two counting systems were used, one a sodium iodide crystal close to the source, the other a group of crystals to obtain a large area, operated at a vertical distance of about 75 ft. A plastic bag containing helium was placed in the space between the source and the distant counters to reduce the absorption of the 14 keV. γ-ray. The transducer was operated sinusoidally, and the counts from the two counting-channels were switched into two pairs of counting systems by mercury switches, so that the pulses obtained with the source moving up were counted separately from those obtained with the source moving down. A dead time was provided to eliminate counts obtained during the switch-over period. The source on its transducer and the large counter were interchanged at intervals so that the expected sign of the asymmetry was changed. Prof. Pound showed a diagram with the asymmetries measured in the two counters in a series of two-hour runs taken in the six days previous to the meeting. The magnitudes of the asymmetries in the two counters fluctuated by larger amounts than could be accounted for by statistical fluctuations, but if averages were taken over all the runs, the measured difference in asymmetry between the counters was of the right magnitude. Prof. Pound emphasized that these results are preliminary, and that the experiments are being continued.

The second paper was read by Dr. T. E. Cranshaw and described a similar experiment performed in the Nuclear Physics Division at Harwell in collaboration with Dr. J. P. Schiffer and Dr. A. B. Whitehead. In this experiment, a 30-mc. source was mounted on a moving-coil transducer also operated sinusoidally. A gramophone pick-up was placed so that the needle was moved by the rod holding the source, and the output from this pick-up enabled the movement of the source to be monitored continuously. No dead-time was provided, the argument being that if the change-over was made when the source was moving slowly, the measured asymmetry is insensitive to the exact instant of switchover. Only one counter, a proportional counter of 6 in. diameter filled to ¼ atm. with krypton was used. Krypton is a particularly suitable gas, because it has an absorption edge just below the 14-keV. line, and so a comparatively low pressure can still give a high efficiency. Two counting channels were used, one fed from the proportional counter, and one fed with pulses from a pulse generator, and both were switched simultaneously in phase with the velocity of the source. The counts from the pulse generator were used to correct the counts from the proportional counter for inequalities of the time intervals. The results from two runs of counting were given, the source being always above the counter with an evacuated tube separating the two. In each run, cycles of four operations, including reversing the phase of the driving current in the transducer, were performed so that each part of the counting apparatus was used to count all four sets of pulses. In this way asymmetries of the apparatus were balanced out. In the first run at 12·5 metres, 30 cycles of 8 hr. each were carried out, and the asymmetry obtained corresponded to a Doppler velocity of $3{\cdot}9 \times 10^{-5}$ cm./sec. In terms of the expected shift, this gives the result $0{\cdot}96 \pm 0{\cdot}43$. The error quoted is that given by statistical fluctuations, and a χ^2 test on the separate 8-hr. runs showed deviations not appreciably greater than could be expected from the statistics. The second run at 3 m., consisting of 8-hr. cycles, was performed as a somewhat redundant test to ensure that the cycling procedure did indeed eliminate systematic errors. The average of these runs was of the expected magnitude, but the fluctuations in the individual points were about twice as large as those expected from statistics. This was attributed to the effects of the increased counting rate with the source at such a short distance from the detector. These experiments will also be continued with a stronger source to obtain a more precise result.

The third paper was read by Dr. J. P. Schiffer, who is a visitor at Harwell on a Guggenheim Fellowship from the Argonne National Laboratory, and described an experiment also carried out at Harwell on a rotating system in collaboration with Drs. H. J. Hay, T. E. Cranshaw and P. A. Egelstaff. The apparatus consisted of a source of iron-57 deposited on an iron cylinder 0·8 cm. in diameter, clamped rigidly between two circular 'Dural' plates of 6 in. diameter. At a radius of 6·6 cm. near the circumference of the plates, a 'Perspex' strip was fixed carrying an iron-57 absorber. The whole system was rotated at speeds up to 500 cycles per sec., giving accelerations of 65,000 times terrestrial gravity. Any shift in the energy of the γ-rays would be observed as a decrease in their absorption, that is, by an increase in the counting rate. The observed change in counting rate as a function of angular velocity followed the variation predicted by the theory of relativity with approximately 10 per cent accuracy due to counting statistics.

The two types of experiment together constitute an illustration of the principle of equivalence. The first type is the only one involving real gravitational fields and this by itself constitutes a test of a prediction of this principle. The rotor experiment can be understood within the framework of special relativity alone. It is, however, the first experimental test of a relativistic effect in a rotating, and therefore accelerated, system of co-ordinates. Theoretical physicists are not completely agreed about the significance of this latter experiment.

T. E. Cranshaw
J. P. Schiffer*

* John Simon Guggenheim Fellow, on leave from Argonne National Laboratory, Lemont, Illinois.

OBITUARIES

Dr. Oliver E. Buckley

Dr. Oliver E. Buckley died on December 14. He will be remembered for many things in the science and technology of electricity. His early years were notable for researches on such diverse topics as the Hall effect, ionization manometers, magnetic materials and submarine cables. His later years were occupied with important government advisory committees and boards. In between came an outstanding but quietly accomplished achievement that will increase in stature as the years go by. This was his work in assuring that basic research will have a secure place in the industrial laboratories of the future. It was he who took over from Dr. F. B. Jewett, the first president of the Bell Telephone Laboratories, a group of scientists who had already given promise of what basic research could do for an industry. Dr. Buckley saw and appreciated this promise. It was he who protected this group from pressures to digress into development. It was he who made it into a recognized function of the Laboratories' activities. It was he who turned it over in mature form to his successor, Dr. Mervin J. Kelly, when he retired from the presidency of the Laboratories in 1951 and as chairman of the Board in 1952.

Dr. Buckley was born in Sloan, Iowa, in 1887. He received the B.S. degree from Grinnell College in 1909 and the Ph.D. from Cornell University in 1914. He immediately joined the Western Electric Company as a research physicist. He soon developed an ionization manometer which was an important tool in the early work on vacuum tubes. Taking an interest in new magnetic materials, he was quick to recognize possibilities in the use of permalloy to increase the speed of telegraph transmission on submarine cable. When perminvar came along, he envisaged a submarine cable capable of transmitting human speech. This project was carried far enough to show that it was quite practical from a technical point of view. The economics of the times determined its fate. Dr. Buckley's interest never flagged, however. In 1932 he started a group working on a multichannel system employing repeaters powered at relatively low voltage with current sent through the cable itself. While this project was never completed in the original form, the work done on it was basic to the cables that now are in operation carrying many conversations across the Atlantic, to Alaska, to Hawaii and soon across the entire Pacific.

When the Western Electric Company's engineering department became Bell Telephone Laboratories in 1925, he was appointed assistant director of research. In 1933 he became director of research and in 1936 executive vice-president. He served as president from 1940 until 1951 and as chairman of the Board until he retired in 1952. During this time many technical advances were made. These included the wave-guide, the transistor, information theory and applications including plastic cable sheaths, automatic message accounting, nation-wide dialing, and micro-wave relay networks.

In the course of his career, Dr. Buckley received many honours and served on a number of important committees. He was a member of the General Advisory Committee of the Atomic Energy Commission, 1948–54, was appointed by the President to be the first chairman of the Science Advisory Committee, and was a member of the National Inventors' Council. He received the Medal for Merit, the nation's highest civilian award, in recognition of his contribution during the Second World War. He was a Fellow of the American Physical Society, the American Association for the Advancement of Science, the Acoustical Society of America, the American Academy of Arts and Sciences, and the American Institute of Electrical Engineers, of which he was vice-president during 1946–48. He was a member of the U.S. National Academy of Sciences, the New York Academy of Sciences, the Franklin Institute, and the American Philosophical Society, of which he served as vice-president in 1954–55.

In the face of these and other achievements, honours and activities, Dr. Buckley retained his characteristic friendly enthusiasm and love for people. He knew a great many of them, and knew how to work with them. To-day, more and more, we see industrial laboratories engaged in basic research. A large share in promoting this belongs to Dr. Buckley. By example in his own laboratories, by logic and inspiring discussion on boards and in committee meetings, and by personal contacts he made people see the need for this type of research and the harvests to be reaped from its cultivation. He has sowed well. The reaping will increase with the years.

F. B. Llewellyn

Captain Guy Gardner

Had he lived a little longer, Captain Guy Gardner's name would have been better known to archæologists in Great Britain than can be the case before the important record, in book form, of his prolonged excavations at the great proto-historic site of Mapungubwe in the Transvaal is available. His death in Johannesburg on Christmas Eve, aged seventy-eight. emphasizes the evils of over-delay in publication, in this case beyond the author's control. For the work was done in six consecutive seasons between 1935 and 1940, and written up during and after the ensuing war years. Its speedy publication is surely now a duty imposed on academic bodies in the Union of South Africa with or without a Government subvention.

Gardner was not a professional archæologist, and, indeed, had his first taste of field-work with me in the Faiyum in 1927–28. But though farming in Natal had been his livelihood ever since he settled in South Africa as a young man, he had loved history and archæology, particularly classical, all his life ; and in the Graeco–Roman remains of my Faiyum concession he rejoiced in his introduction to the reality of the beloved. "The Desert Fayum", Chapter 31, contains his unaided report, illustrated by plans and by drawings of pottery which at the time were the first substantial groups known securely dated to the reign of Ptolemy II.

A more ambitious opportunity followed. The now-famous kopje site of Mapungubwe Hill, accidentally discovered in 1932, had been partially dug by the late Neville Jones and J. F. Schofield in 1934 ; it had disclosed an iron-age Bantu culture, rich in gold

ornament, yet the work of predominantly non-negroid people. This presented unresolved archæological difficulties of interpretation and date manifest in the ensuing publication, "Mapungubwe: Ancient Bantu Civilization on the Limpopo".

Continued research was obviously imperative. General Smuts in a London meeting urged my acceptance of the task; but my reply was to the effect that, on Zimbabwe experience, I preferred passionless archæology. South Africa, I felt, should be encouraged to produce its own excavators. The choice fell on Guy Gardner, who devoted unreservedly his time and energies to six seasons of arduous work, with only occasional European help. Were it not for his letters home, and for his published presidential address to the South African Archæological Society in 1958, and articles in the *South African Archæological Bulletin*, little would be known of his results or his opinions upon them; and even so, obscurities abound for lack of detail. In bare outline we know of his examination of cemeteries and deeply piled middens at the foot of a kopje named Bambandyanalo adjacent to Mapungubwe Hill. The culture, characterized by fine pottery and unusual burial ceremonials, is not that of Jones's and Schofield's people on Mapungubwe, though both populations fall into the Boskopoid–Bush category of South African anatomists, which Gardner terms 'Proto-Hottentot'. His excavations, when published, will have the great advantage over the earlier ones in that carbon-14 samples will introduce fixed dates (within their own wide margins of uncertainty) both for the 'gold users' of the Hill and for the less opulent but more interesting people of Bambandyanalo, who may be at least a thousand years old.

Guy Gardner's meticulous methods of excavation and record, graphic as well as verbal, are a guarantee that a mine of hitherto unknown information awaits impatient readers of his *magnum opus* to be.

G. Caton Thompson

NEWS and VIEWS

Physiology at Bedford College, London: Prof. Margaret Murray

The retirement of Prof. Margaret Murray from the chair of physiology at Bedford College prematurely because of ill-health is a great misfortune to many. She has been associated with Bedford College for forty-two years, as a student when she gained first-class honours in physiology and as demonstrator and as lecturer in biochemistry until in 1947 when she became professor and head of the Department. More than anyone else she has been responsible for the steady progress of physiology for science students in London, and the University owes her much for her services. She has been not only a member of the Boards of Studies in Physiology, and of Biochemistry, but also served on the Board of the Faculty of Science and on innumerable special committees, to which she always gave most conscientious attention. She was chairman of the Special Advisory Board on Dietetics, Nutrition and Household Service for many years and was a member of the Committees of the Medical Research Council on Dental Diseases and on Goitre. She has been a member of the Committees of the Physiological and Biochemical Societies and on the Editorial Board of the *Journal of Physiology*. Her opinions, if sometimes dogmatic, were clear, unafraid, to the point and always respected. Prof. Murray has a wide field of interest on the biochemical side of physiology. Her researches have earned her a high reputation in the scientific world, for her painstaking accuracy is known to all. Her intercollegiate classes on "Blood" were a model of what such classes should be, involving as they did an enormous amount of preparation. In later years she has taken a special interest in the prevention of dental decay by fluorine and its possible hazards, and in the prevention of goitre in hot countries by the use of sodium iodate. In 1937 she was awarded the D.Sc. of the University and now she has been granted the title of professor emeritus.

Prof. W. F. Widdas

Dr. W. F. Widdas, reader in physiology in King's College, London, has been appointed to succeed Prof. Murray. He qualified in medicine in Durham in 1938 and after a short time in practice volunteered for the Royal Army Medical Corps. He served with the British Expeditionary Force until Dunkirk and later held staff appointments at Northern Command and at the War Office with the rank of major. In 1947 he joined the staff of Prof. A. St. G. J. Mc. Huggett at St. Mary's Hospital Medical School, where he worked with a team on fœtal physiology and rapidly became senior lecturer. Dr. Widdas's special interest has been the physico-chemical aspect of physiology, with special reference to the transfer of substances across animal membranes, and he has made notable contributions in this field. He was awarded the Ph.D. (London) in 1953 for a study of the kinetics of glucose transfer into the red cells of adult and fœtal animals, and in 1958 the D.Sc. for his further researches. In 1955 he moved to King's College, where he became reader in 1956. There he has not only been a very active teacher of large classes of medical and science students but has also conducted a very popular intercollegiate course in cell permeability for the B.Sc. (special). He has continued his studies on the red cell and by a most novel method extended his work to cardial muscle, which he has shown loses water during contraction. It is of interest that Dr. Widdas is the twelfth member of the staff of the Department of Physiology of King's College to become a professor in recent years.

Scientific Department of the National Gallery: Mr. F. I. G. Rawlins, C.B.E.

Mr. F. I. G. Rawlins, scientific adviser and deputy keeper at the National Gallery, will retire at the end of March after twenty-five years service. After graduating at Cambridge (Trinity College) and research at the University of Marburg, he held a number of posts at Cambridge where he was also engaged in research in infra-red spectroscopy. He was the first vice-president and secretary-general of the International Institute for the Conservation of Museum Objects, and is technical director of the Central Council for the Care of Churches and a member of the Cathedrals Advisory Committee. He was

the first scientific adviser to the National Gallery, and from very small beginnings he has built up a department which has made notable contributions in its special field. Mr. Rawlins was responsible for the arrangements for the transport of the pictures from the Gallery at the outbreak of war and for providing them with a place of refuge in a quarry in Wales where they were stored under carefully controlled conditions of temperature and humidity. He was successful in securing a grant from the Nuffield Foundation for scholarships at the Gallery. The scholarships proved of such value that the Treasury has assumed responsibility for these posts. The research that has thus been initiated by him at the National Gallery and carried on under his direction may well lead to fundamental improvements in methods of conservation. Mr. Rawlins was made C.B.E. in this New Year Honours.

Mr. R. H. G. Thomson

MR. R. H. G. THOMSON, who succeeds Mr. Rawlins at the National Gallery, has been research chemist (senior scientific officer) there since 1955. Born in 1925, he was educated at Charterhouse and Magdalene College, Cambridge, where he obtained a first-class in both parts of the Natural Sciences Tripos. He stayed on to read English, before joining Dr. Charles Singer in the preparation of "A History of Technology" to which Mr. Thomson was himself a contributor. He is a *rapporteur* of the Scientific Laboratories Committee of the International Council of Museums, and presented a paper at its Copenhagen meeting in 1959 entitled "Picture Varnishes To-day". This aroused considerable interest, and marked substantial progress in a highly specialized field. It is a major theme for further fundamental research in the National Gallery Laboratory. Mr. Thomson has recently become editor of *Studies in Conservation* (the journal of the International Institute for Conservation of Historic and Artistic Works) which brings him into touch with a wide circle of investigators, both in Great Britain and abroad. He is assured of the best wishes of friends and colleagues as he enters upon his new responsibilities.

Geological Society of London Awards

THE following awards for 1960 of the Geological Society of London have been announced: *Wollaston Medal* to Prof. C. E. Tilley, professor of mineralogy and petrology in the University of Cambridge, for his distinguished researches in mineralogy and petrology especially with relation to metamorphism; *Murchison Medal* to Dr. A. G. MacGregor, formerly assistant director of H.M. Geological Survey (Scotland), for his work in vulcanology and petrology and problems of the metamorphic rocks in Scotland; *Lyell Medal* to Dr. Doris Reynolds for her work on the rocks of Northern Ireland and her contributions to petrogenesis; *Prestwich Medal* to Sir Vivian Fuchs in recognition of the geological work of the expeditions which he has led in many parts of the world; *Wollaston Fund* to Mr. M. A. Calver, of H.M. Geological Survey, for his work on faunas of the Coal Measures, especially of the North of England and the Midland Valley of Scotland; *Murchison Fund* to Dr. D. V. Ager, lecturer in palæontology in the Imperial College of Science and Technology, for his researches on brachiopods and on Mesozoic stratigraphy; a *moiety of the Lyell Fund* to Dr. J. D. Lawson, lecturer in geology in the University of Birmingham, for his work on the stratigraphy and palæontology of the Ludlow rocks of the Welsh Borderland; *another moiety of the Lyell Fund* to Dr. R. H. Cummings, lecturer in geology in the University of Glasgow, for his researches on the morphology and evolution of the Carboniferous Foraminifera.

British Scientific Attachés

IN a written reply to a question in the House of Commons on February 3 regarding scientific attachés, the Minister of Education, Sir David Eccles, as representing the Minister for Science, said that five scientific attachés are now attached to British embassies abroad. The attaché at Washington is accredited also as scientific adviser (civil) to the High Commissioner in Ottawa. The attaché at Paris is accredited also to Berne and carries out liaison duties in Italy and the Benelux countries. The attaché at Bonn is accredited also to Vienna. The attaché at Stockholm is accredited also to Copenhagen and Oslo, and carries out liaison duties in Finland. In addition, a scientific adviser to the High Commissioner for the United Kingdom is stationed in Delhi. Attachés or advisers are thus accredited to eleven United Kingdom embassies or High Commissioners' offices.

Appointment of Scientific Officers for United States Embassies

THE U.S. Department of State has announced the appointment of seven additional scientists for its science programme. Three of those appointed are assigned as science officers in New Delhi, Buenos Aires and Rio de Janeiro, four as deputy science officers in London, Stockholm, New Delhi and Tokyo. The men selected are: Earnest Watson, dean of the faculty, California Institute of Technology, as science officer for New Delhi; Neal Weber, professor of zoology, Swarthmore College, as science officer for Buenos Aires; Harry W. Wells, chairman, Upper Atmospheric Section, Carnegie Institution of Washington, as science officer for Rio de Janeiro; John B. Bateman, United States Army Chemical Corps, as deputy science officer for London; William Littlewood, United States Navy Hydrographic Office, as deputy science officer for Stockholm; David C. Rife, International Co-operation Administration Adviser to the Government of Thailand, and formerly professor of zoology, Ohio State University, as deputy science officer for New Delhi; and Marshall Crouch, professor of physics, Case Institute of Technology, as deputy science officer for Tokyo. This is the second series of appointments to be made for the science programme of the Department of State under Wallace R. Brode, science adviser to the Secretary of State. The first appointments assigned science officers to U.S. Embassies in London, Paris, Rome, Bonn, Stockholm and Tokyo (see *Nature*, **183**, 15; 1959).

Anglo-American Space Research

QUESTIONED in the House of Commons on February 15 regarding the Government's future policy towards space research, the Minister of Aviation, Mr. Duncan Sandys, as representing the Minister for Science, said that the plans for Anglo-American co-operation referred to in the Prime Minister's statement of May 12 and of the Minister of Supply's statement of July 29 were developing satisfactorily and we were proceeding with the preparation of the instruments to be carried in the first satellite. Vertical soundings

and experiments in the ionosphere were being carried out in co-operation with Canada and Australia and all Commonwealth countries had been invited to give suggestions on all aspects of the programme and were being kept fully informed of progress. Britain was also going ahead with design studies of the modifications which would be needed to allow Blue Streak and Black Knight to be used for launching satellites and meanwhile Britain would make any use she could of test firings to obtain further information about conditions in the upper atmosphere.

U.S. Planning Committee on Planetary Sciences

The Executive Committee of the American Geophysical Union of the National Academy of Sciences has formed a Planning Committee on Planetary Sciences. The purpose of the new committee will be to accommodate the increasing number of American Geophysical Union members engaged in planetary and space research by expanding the Union's activities in this field. The following committee officials are announced: Homer E. Newell, jun. (deputy director, Office of Space Flight Programmes, National Aeronautics and Space Administration) as chairman; Robert Jastrow (Theoretical Division, Goddard Space Flight Center) as secretary. Other members of the committee are Philip H. Abelson, Leroy R. Alldredge, J. W. Chamberlain, Thomas Gold, J. G. F. MacDonald, Hugh Odishaw, Allen Shapley, E. H. Vestine, Harry Wexler and Charles A. Whitten.

School Children in Africa

More than half the world's children are now in school. Unesco figures, collected over a three-year period, estimate that there are approximately 550,000,000 children aged 5–14 years in the world to-day, but only 300,000,000 of them are in school. During the early 1950's, the world passed the half-way mark in getting school-age children into classrooms. The enrolment figure was 48 per cent in 1950 and 55 per cent in 1954. Many countries to-day still show low enrolment figures, especially in Asia and Africa; but it is in these countries that enrolments are increasing the most. In the Belgian Congo, for example, the number of primary school pupils rose from 145,000 in 1930 to 1,060,000 a quarter of a century later (*South Afr. J. Sci.*, **55**, No. 7; July 1959). Enrolment in Ghana rose from 55,000 in 1932 to 508,000 in 1954. Girls' education is no problem in Basutoland where the difficulty lies in getting more boys into school. Owing to the economic pattern of Basuto life, where boys are traditionally herdsmen, girls outnumber boys in class by two to one.

Contemporary Physics

Two numbers of the new journal, *Contemporary Physics*, have so far appeared, and there are excellent articles in both (Taylor and Francis, Ltd., 18 Red Lion Court, London, E.C.4. 27*s*. (4 dollars) per vol. of six parts; 5*s*. (75 cents) per part). The journal aims at interpretation and review—to maintain the highest level of scientific content, but at the same time to present the content in such a way that the young graduate, and the teacher needing a refresher course to bring him up to date with modern developments, may understand without undue difficulty. The editor has succeeded in obtaining first-rate authors to write articles on interesting and topical subjects, but generally the standard is rather high and the text too burdened with fearsome mathematics and frightening graphs. There are exceptions, of course, like the articles on skidding friction by Barbara Sabey, of the Road Research Laboratory, and on diamond by Prof. S. Tolansky; but the reader turning with expectation to Sir Harrie Massey's article on space research may well be put off by the fact that it contains no illustrations at all. It is conceivable that it will be the university teacher and the more advanced physicist who will spend many enjoyable hours reading *Contemporary Physics*. The article, "Molecular Beams", by Prof. O. R. Frisch, for example, is an excellent introductory survey to the subject which will neither dismay a postgraduate research physicist working in some other field of physics, nor give him the impression that the author is talking down to his reader.

Specific articles in both numbers are devoted to historical or educational aspects of physics. In No. 1 (October 1959), Dr. C. Kellner discusses Alexander von Humboldt and the organization of international collaboration in geophysical research; Mr. H. V. Beck, the selection and design of experiments for alternating current teaching; and in No. 2 (December 1959), F. Lester describes a course in the physical sciences for teachers in training; Sir John Cockcroft, the newly founded Churchill College; and L. R. B. Elton the vexed question of a clearing-house to help students to find admittance to honours courses in physics. *Contemporary Physics* deserves to do well; perhaps, without departing too much from its initial standard, its content could be made more appealing in form and less suggestive of another learned society journal, so that it may reach the wider public for which it is intended and which it richly deserves.

Botanica Marina

Scientific periodicals are already so numerous that the appearance of a new one inevitably arouses misgivings. There does, however, seem to be a very good reason for the birth of *Botanica Marina*, the first number of which has recently appeared (Cram de Gruyter and Co., Hamburg, 1959). As Prof. Drescher-Kaden points out in the introduction, classical botany has for hundreds of years been essentially *Botanica Terrestris*, but recently both planktonic and benthic marine algae have been attracting more attention than formerly. Their intrinsic interest is great and their practical importance large and increasing, so it is obviously desirable to have a journal for the publication of work on them. The editorial committee, which is truly international, must inspire faith in the future of this journal, while the contents of the first part give an indication of the range of studies to be included—cell physiology, chemistry, statistical ecology and what might be called 'straight phycology'.

It would be interesting if in some future number we could be given an account of the debt botanists owe to the work of zoologists and chemists in the investigation of marine phytoplankton. It must be considerable. *Botanica Marina* looks like becoming an essential periodical for every library with marine interests and a useful one for such others as can afford it.

Technological Education and the Nuffield Foundation

The Nuffield Foundation has made an imaginative and constructive approach to senior colleges of technology which should do much to raise the standards of technological education in Great Britain. In a letter to the principals of those colleges which provide

advanced sandwich courses, the director, Mr. Leslie Farrer-Brown, has indicated that the Foundation would be prepared to consider any requests for financial help which would lead to improvement in the quality of technological education provided by the colleges. The Foundation in no way seeks to limit the way in which senior colleges of technology will develop and hopes that each will grow in a manner in which it can best serve the needs of industry. As education for technology must be given parity in technical institutions and in industry, there is room for considerable experiment about the ways in which this should be organized. So, too, with the teaching methods which have yet to be evolved. Among a number of tentative proposals, Farrer-Brown indicates that "perhaps, in suitable cases, the attainment and maintenance of high standards of teaching and the complementary training in industry, might be fostered if teachers of their colleges and their associated educational staffs in industry had the opportunity to study educational methods at technological institutions overseas". In the first place principals have been invited to submit informal proposals.

Leverhulme Trust

THE second report of the Leverhulme Trustees classifies the awards made during the first twenty-five years of the activities of the Leverhulme Trust Fund (The Leverhulme Trust. Analysis of Grants 1932–1955: Second Report. Pp. 18. London: The Leverhulme Trust, 1959). Of the total of £854,358, £179,400 was awarded to universities and centres of higher education; £84,000 to learned societies, research institutions, etc.; £132,700 to schools; £3,600 to archæology; £54,000 to the arts; £28,300 to architecture and town planning; £115,300 to medical research; £58,600 to trade charities; £26,400 to trade educational grants; £40,800 to miscellaneous grants, with £131,258 on the Advisory Committee, individual research awards and grants. No capital grants or contributions are made to appeals of a general nature. Long-term grants are, as a rule, excluded, and broadly speaking the Fund goes mainly to the furtherance of research in the universities, teaching hospitals and other centres of higher education, the Trustees' primary concern being to foster new ideas which may lack support from other sources.

Scientific Research in Schools

THE Committee on Scientific Research in Schools which was established by the Council of the Royal Society on March 7, 1957, reports that interest shown by schools in undertaking research projects has increased and all the research projects originally supported by Shell Research, Ltd., have now been transferred to the Committee. There are now forty-eight schools undertaking scientific research; some of the schools have more than one project under way and a total of fifty-seven separate research projects are now being administered compared with twenty-five projects last year. Various requests for advice have also been received from another two schools and these are under consideration. During the year a sum of more than £2,000 has been granted to those carrying out research. The Council of the Royal Society has provided a sum of £2,000 and Shell Research, Ltd., have given an additional £127 10*s*. in addition to their support of research in two schools. A scheme has also been arranged with the Director of the National Physical Laboratory, whereby, during the summer holidays, schoolmasters selected by the Science Masters' Association can visit the Laboratory and carry out some scientific work there. All research projects with which the Committee is dealing are carried out with the specialist advice and assistance of Fellows of the Royal Society and by others who have agreed to act as advisers. The Committee has been stimulated by the continued and growing response to its scheme and looks forward both to receiving further requests from schoolmasters and schoolmistresses and to the continued support of the Fellows of the Royal Society and others in acting as advisers and assistants in the extension of its activities. This enterprising scheme should do much to raise the standard of science teaching in schools.

The Geological Survey of Great Britain

THE Summary of Progress of the Geological Survey and Museum for 1958 records much work in the field and offices. With regret, however, it directs attention to the shortage of trained cartographical draughtsmen, who are so important in the preparation of the publication of geological maps. This regret is indeed shared by many who have to wait for such long periods to see the results of the field and subsequent reports that have been prepared by the geologists. Primary survey has been carried out in several areas and revision was continued mainly in areas of economic importance, particularly the coalfields. Statistics of mapping are given for the quinquennium. Exploratory boring is now an important part of the work of the Survey and the results of various geophysical methods are interesting. The Survey has continued as geological consultant to the United Kingdom Atomic Energy Authority, and an airborne radiometric survey of the remainder of Cornwall, much of Devon and part of Somerset has been undertaken. The Museum attracted nearly 390,000 visitors, and re-organization of the reserve mineral and economic collections and research upon them continued.

A Technique for Capturing Birds and Mammals

CAPTURING large numbers of wild birds and mammals for the purpose of marking the animals for ecological and behavioural studies is usually a difficult task. A paper by Ronald F. Labisky describes a technique which was found effective for capturing pheasants (*Phasianus colchicus*) and certain other animals by using bright lights at night to blind them temporarily ("Biological Notes", No. 40. Natural History Survey Division, University of Illinois). The capturing of pheasants by night-lighting, also called 'jack-lighting' or 'shining', has been employed in practical game management since the late 1920's. Oscar Johnson reported that workers in South Dakota captured about 10,000 pheasants during the winter of 1926–27, and 12,000 pheasants during the winter of 1929–30, by 'shining' roosting birds with motor-car headlights. More recently, workers in Idaho, South Dakota, Nebraska and California have employed night-lighting in capturing pheasants. In Illinois, one of the problems in pheasant research has been that of capturing and marking a large enough number of wild pheasants to permit investigations of behaviour, population dynamics and movements. While bait trapping of wild pheasants is successful in many States, it is difficult, or frequently impossible, in east-central Illinois during autumn and winter; this difficulty is probably the result of an abundant year-round food supply and moderate winter weather.

In order to facilitate the pheasant research programme in east-central Illinois, a mobile night-lighting rig was designed. The night-lighting apparatus was also found to be useful for observing and studying the behaviour of animals during the hours of night. This study technique is particularly valuable when many animals have been marked for individual recognition.

Stone-fruit Viruses

In serological and physical studies of stone-fruit viruses, R. S. Willison, M. Weintraub and J. H. Tremaine have reported that strain *Y.4* of the cherry yellows virus was purified from cucumber by four methods (*Canadian J. Bot.*, 37, No. 6, 1157; 1959). These differed mainly at the clarification stage. Two of the methods, which are given in detail, yielded infective preparations. The virus was usually inactivated by freezing. Ultra-violet absorption spectrum analysis, and particle-size and sedimentation-rate determinations, supported by infectivity tests, indicated that, in these infective preparations, the virus replaced most if not all of the components found in comparable extracts from healthy sources. The agar-gel diffusion technique was used for precipitin tests. Virus antigen from cucumber sources reacted with homologous antiserum or with antiserum derived from infected cherry petals and vice versa.

Letters written by Frédéric or Irène Joliot-Curie

P. Biquard, writing on behalf of the Association Frédéric et Irène Joliot-Curie, Institut du Radium, 11 rue Pierre-Curie, Paris 5e, asks if readers of *Nature* possessing letters written by Frédéric or Irène Joliot-Curie would inform him. It is one of the aims of this Association to publish such correspondence, and it would like to receive such letters, or photocopies. The letters will only be published if their owners give the necessary authorization.

Classical Languages in Oxford Entrance Requirements

Congregation at Oxford has approved a scheme proposed by the Committee for Admission to the University for new entrance requirements. The position of Latin and Greek was the topic of widest interest in the new proposals, under which Latin would no longer be essential. The Committee for Admission recommended exemption from Latin for those who have a pass at Advanced Level in a General Certificate of Education examination or a college award in mathematics or science. This proposal was accepted, and a statute embodying the decision will be brought before Congregation next term. To become effective, the statute will require a two-thirds majority.

Colombo Plan Appointments

In a written answer in the House of Commons on February 12, the Minister of State for Commonwealth Relations, Mr. R. Thompson, stated that in consultation with the Government of India, the United Kingdom Government is arranging to recruit and provide, under the Technical Co-operation Scheme of the Colombo Plan, ten members of the professorial staff of the College of Engineering and Technology in the University of New Delhi, and two science workshop supervisors. It is intended that the professorial appointments should be for average periods of five years.

Announcements

Sir Harry Melville, secretary of the Department of Scientific and Industrial Research, will formally open the new laboratories of the Coil Spring Federation Research Organization in Sheffield on March 24 (see *Nature*, 184, 777; 1959).

The twenty-fourth meeting of the European Federation of Chemical Engineering will take the form of an International Symposium on Distillation, which will be held in Brighton during May 4–6. Further information can be obtained from the Institution of Chemical Engineers, 16 Belgrave Square, London, S.W.1.

Under the auspices of the Laboratory Animals Centre, a symposium on the Provision of Animals for Cancer Research will be held at the Royal Society of Medicine on May 23. Further information can be obtained from the Secretary, Laboratory Animals Centre, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey.

The Department of Mechanical, Civil and Chemical Engineering, Royal College of Science and Technology, Glasgow, is organizing a symposium on Nuclear Reactor Containment Buildings and Pressure Vessels during May 17–20. Further information can be obtained from the Secretary of the Royal College of Science and Technology, Glasgow, C.1.

The Royal Microscopical Society is arranging a two-day meeting in conjunction with the Electron Microscopy Group of the Institute of Physics, to be held in Leeds on March 31 and April 1, to discuss "Co-ordination of Light and Electron Microscopy". Application forms can be obtained from the Secretary, Royal Microscopical Society, Tavistock House South, Tavistock Square, London, W.C.1.

The seventh international congress of the European Ceramic Association is to be held in London during May 23–28. The purpose of the conference is to establish opportunities for the discussion of common interests and problems between specialists from all parts of western Europe. Further information can be obtained from the organizing secretary, Mr. H. O. Cox, 36 Victoria Street, London, S.W.1.

The Chemical Society, the Institute of Petroleum, the Royal Institute of Chemistry and the Society of Chemical Industry are jointly organizing a symposium to be held in the Chemistry Lecture Theatre, the University of Manchester, on March 24, on the Industrial Chemistry of the Lower Olefines. Further information can be obtained from Dr. P. J. King, Department of Chemical Engineering, Manchester College of Science and Technology, Sackville Street, Manchester 1.

Dr. Marie Boas's book "Robert Boyle and Seventeenth Century Chemistry" (Cambridge University Press, 30*s*. net; see *Nature*, 183, 850; 1959) has been awarded the Pfizer Prize of the History of Science Society for the best book on the history of science by an American author published in 1958. "The Rotation of the Earth", by Prof. W. Munk and Dr. G. J. F. MacDonald of the University of California, has been awarded the Monograph Prize of 1959 of the American Academy of Arts and Sciences for a work on a physical or biological subject. This book, a Cambridge Monograph on Mechanics and Applied Mathematics, will be published by the Cambridge University Press this autumn.

DEPARTMENT OF CHEMICAL TECHNOLOGY, UNIVERSITY OF BOMBAY

THE Department of Chemical Technology of the University of Bombay has just celebrated its silver jubilee, and it is thus an opportune time to review briefly the development of this Department and its impact on Indian industries. Its foundation may be largely attributed to the vision and enthusiasm of Sir M. Visvesvaraya, who, as chairman of a Government of India committee, recommended as long ago as 1921 that a faculty of technology be instituted in the University of Bombay and that a College of Technology be established in Bombay City. It is probable that the practical difficulties associated with so large a scheme delayed its fulfilment, for it was not until 1930 that the matter was raised again in a more modest way, with the result that in 1934 the University started two new two-year courses, one in textile chemistry and the other in chemical engineering, to each of which twenty chemistry graduates might be admitted annually. The choice of subjects was a wise one: chemical engineering for its general applicability to all industries, and textile chemistry because of its value to the immense cotton industry of the Bombay Presidency. The first graduates were well received by industry, and the demand soon became far greater than the supply.

Moreover, many private individuals and associations, seeing that this was the beginning of a new era in industrial development, made handsome contributions for the endowment of chairs and readerships, or for the establishment and equipment of specialized laboratories. These have amounted so far to £280,000, and in conjunction with large government grants have made possible the erection on a 14-acre site of a group of laboratories of the most modern type on a unit basis, which allows extension or subdivision with a minimum of disturbance. Special features are the large quantity of small-scale plant and high-class instruments intended mainly for research work. There are a well-equipped workshop and a spacious drawing office in which all students have to work, and the library contains about 8,000 modern technical works and more than 200 periodicals to which readers have free access.

The two sections with which the Department started were originally housed in part of the Royal Institute of Science, which gave little room for expansion; but as soon as the success of the project was assured, preparations were made to build the new laboratories and to add more sections to the Department. Starting in 1943 when the laboratories were ready, new branches were gradually added, and there are now seven two-year courses of study open to chemistry graduates which lead to the degree of B.Sc. (Tech.). These are textile chemistry, and the technology of pharmaceuticals and fine chemicals, food, intermediates and dyes, oils, fats and waxes, plastics, and pigments, paints and varnishes. Chemical engineering is now a four-year course, starting from intermediate, for which the degree of B.Chem.E. is awarded, and there is also a three-year degree course from intermediate in pharmacy. In addition, emphasis is laid on research and work for higher degrees, and there are now 125 postgraduate students out of a total of about 525. Members of the Department have published in all more than 700 scientific notes, articles and papers, mostly on experimental work conducted in the laboratories. The Department also acts as a test house for the textile industry and undertakes sampling, analysis, the investigation of technical difficulties and more comprehensive schemes of industrial research for outside bodies.

The number of students has increased in a remarkable manner. Starting with 109 admitted during the first five years, it rose to 380 ten years later, and during the past four years it has been 785. The proportion taking a degree is very high, more than 1,500 of the 1,700 admitted having been successful. These graduates are much sought after; up to the end of 1957, the last year for which detailed figures are available, 762 were engaged in the manufacturing industry out of a total of 1,316.

Unfortunately, this healthy expansion is threatened by circumstances which must already be a source of anxiety to the authorities—the almost world-wide problem of staff. In Bombay the chairs of textile chemistry, food technology and chemical engineering are all vacant, and in the last-mentioned section one readership is vacant and one lecturer is on study leave, leaving one reader and a temporary lecturer to deal with a four-year course to which forty-five or even more students are admitted annually. It is evident that adequate teaching of the wide range of subjects contained in the syllabus cannot be maintained without a large increase of staff. Other sections are suffering in the same way, although to a smaller extent, and the trouble has been mitigated slightly by members of the staff of other institutions, seventeen of whom are generously helping by giving part-time assistance. It would seem that a satisfactory state of affairs will not be reached until the Department's own graduates and those of similar establishments have increased in numbers and experience to such an extent that the small percentage who take up teaching (augmented perhaps by higher salaries) will be sufficient to provide an adequate staff.

The effect of the introduction of 1,500 trained scientists with an acquaintance with industrial conditions and a specialist knowledge of certain technical operations into the country, followed by more than 150 every year, cannot fail to exert a profound influence on the economy of Bombay and indeed of the whole of India. When it is considered that a large proportion of these men have, in addition, a research outlook which may lead to important improvements, it is difficult to form any estimate as to the changes which may result. It suffices to say that these changes can be only for the good and likely to give the utmost satisfaction to all who so wisely gave their time and their money to further the cause of technical education. H. E. WATSON

THE BRITISH COUNCIL

THE annual report of the British Council for 1958–59 includes a brief tribute to the late chairman, Sir David Kelly, the customary appendixes, particulars of university interchanges, courses and study tours, etc., arranged by the Council in the United Kingdom or overseas, exhibitions and publications, analysis of estimates and accounts, and an assessment by the director-general, Sir Paul Sinker, of the main tasks confronting the Council (The British Council. Annual Report 1958–59. Pp. vi+114+9 plates. London: British Council, 1959). Over the past five years, the Council's gross income, derived mainly from public funds, has almost exactly doubled, from £3 million to £6 million. Its work is carried out in about seventy countries, now some four-fifths in the developing areas of the world, especially in the Near and Middle East, South and South-East Asia and Africa, rather more than half of this being devoted to the recently independent countries of the Commonwealth and to Colonial territories, and the fifth, devoted to Europe, includes an important extension to the German Federal Republic.

Sir Paul Sinker emphasizes three tasks as of pre-eminent importance: educational work, especially but not exclusively in the teaching of English; the fostering of personal contacts between British and overseas people, especially between opposite numbers in the educational, scientific, professional and cultural fields; and helping to ensure that English reading matter is as widely available as possible in the countries with which the Council is concerned. In educational work he comments that the recruitment of British teachers for overseas posts has been a main point of growth during the past two years, and the Council has been concerned to ensure good salaries and living conditions, reasonable security and opportunity for obtaining a suitable job on return. All this has meant additional work for the Council's representatives overseas, and since 1957 these posts have been increased by twenty-five of an educational character. The filling of university posts has presented special difficulty. As regards personal contacts, besides some 40,000 overseas students in Britain, the Council looks after, every year, some 5,000 senior visitors, and Sir Paul directs attention to the proportionate increase in the number of visitors for scientific and technical purposes and to the very considerable increase in the Council's responsibilities for administration of the United Kingdom fellowships and similar schemes on behalf of the Government and other authorities. More than 2,000 Fellows and Scholars a year are cared for under various schemes, and the Council is also responsible for organizing a smaller but important outward flow of specialist tours from the United Kingdom. On the printed word, Sir Paul Sinker stresses the key importance of the library in a British Council centre and the advances on a wide front which have been made possible by the Government announcement on June 22, 1959, regarding the export of British books, etc. Finally, he refers briefly to the opportunities which arise as Colonial territories attain independence and also to those offered by educational television.

The general survey of the year notes that the Council's responsibilities are likely to be increased as a result of the Commonwealth Educational Conference in July 1959, as it is probable that the Council will be asked to assist in implementing the main recommendations of the Conference. About half the £1·5 million by which the Council's grants have been increased since 1957 has been used to implement the decisions announced in the first White Paper regarding the teaching of English overseas. Eighty teachers have been recruited for specific posts, mainly in India, Pakistan, South East Asia, the Middle East and Africa, and almost double this number should be recruited in 1959–60 and a further seventy teachers supplied by the provision of financial assistance to schools. Besides 100 scholarships awarded to bring overseas teachers to the United Kingdom for training, a further 68 are being awarded in 1959–60, and the Council has been encouraged by the growing interest of British universities in these problems. Qualified lecturers and tutors are needed, however, at summer schools for teachers of English both at home and abroad, and a further problem is the provision of material for English language teaching.

Agreement was reached with the government authorities of several countries, notably Pakistan, to extend full Council services to their students in Britain, and during the year 7,500 overseas students were met on first arrival. Some 101 specialist tours, advisory visits and delegations, included one to Poland by Prof. J. N. Davidson, the first of several visits of biochemists arranged at the invitation of the Polish Academy of Sciences, and another to India by Sir Harry Platt. Prof. R. M. Kenedi, of the Royal College of Science and Technology, Glasgow, was invited to visit Lima Engineering University to lecture, assist in organizing programmes of studies and advise on purchase of equipment, and a British engineering library has been opened in the Institute of Higher Studies, Buenos Aires. Under the Commonwealth Scheme of university interchange, awards were made to permit ten short visits for consultation and lectures and 47 travel grants were made to university teachers and postgraduate research workers: under the foreign scheme 121 short visits and three longer visits were arranged, besides 57 visits under the younger research workers interchange scheme. Of 5,153 overseas visitors in the United Kingdom under the auspices of the British Council, 996 were in science and technology, 1,255 in education, 1,152 in social science, and 974 in medicine, 739 coming from the Middle East, 562 from South-East Asia and the Far East, 347 from Latin America, 1,232 from Central and East Europe, and 953 from West Europe. Of the 7,487 students met on arrival, 459 were found permanent accommodation in Council residences, 310 in university halls of residence and hostels, and 3,215 in lodgings. Transit accommodation on first arrival was arranged for 1,877 in Council residences and in lodgings for 1,178, while temporary accommodation was arranged for another 1,354 in Council residences and 4,686 in lodgings, and 9,749 students enrolled at Council centres, 2,353 attending vacation courses of 7–15 days, 1,340 week-end courses, and 8,778 study visits to factories, museums, etc.

CYCLICAL ACTIVITY IN ENDOCRINE SYSTEMS

COMPARED with members of most learned bodies, Fellows of the Zoological Society of London have always been well served—more so than many have realized—in regard to the collections, the library, and the gastronomic and other facilities that they are privileged to enjoy. Additionally, in recent months, there has begun a most welcome series of one-day symposia which will in future take the place of a proportion of the ordinary scientific meetings of the Society. These symposia are not too narrowly specialist, and they are open to all zoologists whether Fellows of the Society or not.

The second such symposium, on "Cyclical Activity in Endocrine Systems", was convened by Prof. E. J. W. Barrington, and held on December 8.

The first two papers dealt with the cyclical activity of the fish thyroid. Dr. A. J. Matty (Nottingham) pointed out that although recently a number of reports have indicated a seasonal change in the thyroid, most have been derived from histological data. Only in one species has hormonal iodine been determined. Variation from species to species both in degree and time of thyroid activity made few generalizations possible, although it is possible in some instances to correlate a seasonal thyroid change with locomotory and migratory changes. Some fish appear to have a more active thyroid during spawning, and Dr. Matty suggested that, as the gland does not seem directly to stimulate gametogenesis, the relationship is due to the influence of the thyroid on protein metabolism, for example, on gonad growth or the development of viviparous young. The thyroid in fish has been shown to influence growth, metamorphosis and integument development and, as current researches have demonstrated that nitrogen metabolism is influenced by thyroxine, it was suggested that a principal role of the piscean thyroid is the control of protein and nucleoprotein metabolism.

Dr. D. R. Swift (Windermere) made a general survey of the external factors which could affect the thyroid and its demonstrated cyclical activity. He emphasized that we do not know what the epithelial cell height and uptake and loss of radioiodine mean in terms of actual activity of the gland and that it was difficult therefore to compare the results obtained by workers using different methods. Dr. Swift suggested that the thyroid undergoes two periods of high activity, one period coinciding with the maturation of the gonads, with which it may be in fact concerned. The second period of high activity occurs as part of what is called the basic cycle of thyroid activity in these animals. This activity of the gland appears to be inversely proportional to the water temperature. It was suggested that temperature plays a major part in the control of the thyroid, but that the length of the photoperiod may influence the gland at low temperatures. It was stressed that it is the change in the environment which is important. Obviously, in Nature, the fish never experience a constant one.

Dr. P. G. W. J. van Oordt, a welcome visitor from Utrecht, is primarily interested in the Amphibia. He spoke of the influence of internal and external factors in the regulation of the anuran spermatogenetic cycle. He concluded that spermatogenesis depends on two so-called final factors, first (as would be expected) the gonadotrophic activity of the pituitary, and secondly, the sensitivity of the germinal epithelium to gonadotrophic hormone. Each such factor is influenced by one or more 'intermediate' factors. These include, for example, a gonadotrophin secretion-inducing agent produced by the preoptic nucleus of the hypothalamus. The male sex hormone is also influential. In Amphibia with a potentially continuous spermatogenetic cycle this activity is primarily regulated by the external temperature. This basic factor, together with the internal rhythm, determines the seasonal changes in the spermatogenetic activity of Anura with a discontinuous spermatogenetic cycle *sensu stricto*.

Dr. A. J. Marshall (London) next discussed cyclical phenomena and corresponding behavioural adaptations in relation to the environment in birds. While paying due regard to the importance of day-length changes as a regulatory factor, he remarked that if we viewed the avifauna of the world, light is important only in species for which it is important that it should be important. A whole range of widely unrelated species would be extinguished by natural forces if they were compelled to reproduce at any particular phase of the cycle of the Sun. In perennially lush areas, it is often biologically beneficial—and possible—for species to have more than one breeding season per year. There is a fascinating, as yet unwritten, story of the changes in behaviour that have gone hand in hand with the neural and endocrine changes dictated by the environment and natural selection.

The complexity of endocrine cycles in vertebrates was emphasized by Prof. E. J. W. Barrington, who gave illustrations from the work of his Nottingham group to show the difficulties encountered in attempting to demonstrate a one-to-one relationship between a particular cycle and a particular function. He concluded that an essential part of the study of such cycles must be the means by which they are co-ordinated and brought into relationship with the external environment. This involves, in particular, a study of the pituitary and its relationships with the central nervous system. The evolution of the trophic control exerted by the adenohypophysis is one aspect of such a study, and he discussed from this point of view the relationship which has recently been claimed to exist between the adenohypophysis and the endostyle in the ammocoete larva. Results obtained at Nottingham suggest that it is by no means clear that such a relationship is demonstrable on the present evidence. Finally, he considered the problem of the relationship between the hypothalamus and adenohypophysis in teleosts and gave evidence suggesting that (contrary to what has previously been supposed) the vascularization of a particular area of the floor of the infundibulum has at least some of the characteristics of a median eminence, and that glial secretory cells are found in the same area. Identifications of such areas in other fish, and a study of any cyclical variations which

they may show, should shed much light on the regulation of endocrine cycles in the lower vertebrates.

Dr. G. W. Harris (London), who thoughtfully made it a condition of his appearance that he would not have to write a paper, dealt comprehensively with comparative aspects of the functional relationships of the hypothalamus and adenohypophysis. He paid a graceful tribute to the pioneer work of the late F. H. A. Marshall in the field of reproduction physiology.

The final two papers were concerned with invertebrates. Dr. M. J. Wells (Cambridge) gave an account of some of his work on the optic glands and the ovary of *Octopus* and described a hormonal system which regulates the onset of sexual maturity. In cephalopods, maturation of the gonad depends upon release of a protein secretion from the optic glands. These are in turn governed by an inhibitory nerve supply originating in the highest centres of the central nervous system. The optic glands can be activated (causing precocious sexual maturity) by severance of this nerve supply, or by peripheral blinding. 'Ripening' of the oviducts and oviducal glands is independent of ovarian maturation. The system is single-stage; the state of these secondary structures is not under the control of sex hormones. The brain-optic gland system of cephalopods has obvious analogues in chordates and arthropods; the factors determining the convergent evolution of mechanisms for delaying the onset of sexual maturity were discussed.

The final paper was by Dr. D. B. Carlisle (Plymouth) on moulting cycles in Crustacea. The act of moulting in arthropods, said Dr. Carlisle, must not be considered as an isolated event but as the most spectacular moment in a continuous cycle of metabolic activity. Each moulting cycle is basically like the one before it, and many of the stages have now been shown to be under hormonal control. Secretion of the 'moult inhibiting hormone' by the endocrine complex of the eye-stalk promotes the formation of new tissues and prevents the *Y* organ from secreting more than 'marginal' amounts of its hormone. Once this eye-stalk hormone ceases to be secreted the *Y* organ releases a single 'dose' of a moult-promoting hormone. This initiates the preparations for the moult which, once under way, are co-ordinated by a moult-accelerating hormone from the *X* organ. The amount of water taken in at the moult itself is regulated by a further eye-stalk hormone. Many other hormones act upon particular moults and modify them in some way, acting mainly upon the stage of proecdysis and making the succeeding moult in some way unique: the moult of puberty and the moult at which a female becomes furnished with the brooding characters are examples. In the same way that the originally similar segments of the vertebrate body have been progressively and uniquely modified (in, for example, the head) so the originally similar moults of arthropods have been modified, until we have what Dr. Carlisle called 'the startling difference' between the larval and imaginal moults of Lepidoptera.

Prof. J. Z. Young, who was chairman of the invertebrate session, enlarged upon a statement made by the final speaker concerning the complications of modern endocrinology. Prof. Young said that the presence of a multiplicity of hormones was the natural consequence of the fact that they were sent out, broadcast through the body and each needed to be addressed to a particular target. No such multiplicity of chemical mediators was necessary in the nervous system, where addresses were specified by connexions. The discovery of more and more distinct hormones was a sign of the maturity of the subject. Endocrinologists were learning to understand the code with which their complicated system operated.

The symposium concluded with a general discussion and a summary by Sir Solly Zuckerman. The papers will be published soon as one of the *Symposia of the Zoological Society of London*.

A. J. Marshall

AERODYNAMIC CAPTURE OF PARTICLES

A SYMPOSIUM on this subject was held during January 13–14 in the laboratories of the British Coal Utilization Research Association at Leatherhead.

Although this might be thought to be a field of very limited appeal, yet, as evidenced by the wide interests of the fifty scientists present, the impingement of small particles carried in a stream of air and falling on to a solid surface placed alongside or in the way of the flow is a problem which concerns a number of industries, as well as having meteorological implications ranging from the icing of aircraft wings and motor-car windscreens to defence against microbiological warfare.

The wide scope of the applications of this study can be seen by looking through the list of titles offered at this symposium. In 'pure' meteorology one studies cloud formation and collision of raindrops, in applied meteorology the wash-out by rain of particulate matter, whether this be the product of industrial air pollution or of an atomic explosion. A number of cognate problems arise in the laying of mine dusts by water sprays. Then we have the impact of water drops or ice crystals on large moving surfaces such as aircraft wings or turbine blades; when both the obstacle and particles are on a smaller scale we have the action of the gas-mask filter (one of the earliest applications).

The coagulation of particles carried by a stream of air or water is, of course, of great interest and involves many applications where solid or liquid particles have to be transported from place to place, for example, in the industrial process of fluidization.

The basic theory of much of this work stems from Albrecht, who worked out in 1931 the proportion of particles carried by a stream of air which would be intercepted by a cylinder placed athwart the stream. The particles tend to follow the stream lines as they approach the cylinder, but their mass makes them continue in a forthright direction. If their resistance in the stream is given by Stokes's law, that is, direct proportionality to velocity and viscosity, their actual paths depend on this balance between inertia and viscosity in a parameter resembling a particle Reynolds number. This 'particle parameter' has been

used continually in capture problems since (and deserves the attribution of Albrecht number).

The conference, opened by a short address of welcome from Dr. Idris Jones, director of research of the National Coal Board, was divided into three sessions. The first morning covered fundamental aspects, a number of developments of the Albrecht thesis, using modern mathematical devices and including other types of flow around the obstacle than the potential one (H. Herne). Prof. P. R. Owen had devoted a considerable study to the process by which a turbulent wind-stream deposits particles on the roof of a tunnel, while Dr. D. J. Brown had studied the behaviour of particles in the process of froth flotation. Dr. T. Gillespie, who had come from the United States to attend the symposium, described his interesting series of researches on the role of electric forces in collision processes.

The afternoon session was devoted to practical applications of capture techniques. Accounts were given of the deposition experiments on radioactive particles and gases carried out at the Atomic Energy Research Establishment (A. C. Chamberlain); on particulate filters—one of the earliest applications—at the Chemical Defence Experimental Establishment (R. G. Dorman); on fish-curing smokes at the Herring Industry's Research Station (W. W. Foster) and two applications in the aircraft industry, namely, impingement of water drops on a surface at supersonic speeds (D. C. Jenkins and J. D. Booker) and on turbine blades (D. L. Martlew).

In the final session the papers dealt with collisions of particles with rain-drops, and of drops with each other. The difficult calculations on the relative motions of particles of comparable size—when the flow pattern around each contestant must be known—had been made by Dr. L. M. Hocking; but other papers were primarily experimental. A long and careful series of experiments by W. Walton and A. Woolcock and less-extensive ones by Dr. R. G. Picknett involved the measurement of the pick-up by a single water drop on which small particles impacted. Finally, the effect of the efficiency of the pick-up process when the particles are wettable and can be engulfed or pass completely through the water drop was discussed both theoretically (by C. S. Pemberton) and experimentally (by B. Oakes).

At the conclusion of the third session, Mr. W. Walton (National Coal Board) thanked the staff of British Coal Utilization Research Association for offering the symposium the hospitality of its conference rooms and laboratories, the work in which the visitors were able to inspect before leaving.

Lively discussions followed the reading of the papers. The whole proceedings are to be published by Pergamon Press, Ltd. and should form an interesting volume in a field which has not been heretofore covered by a book.

E. G. RICHARDSON

THE SHUTTLE-BOX BEHAVIOUR OF ALBINO RATS DURING PROLONGED EXPOSURE TO MODERATE-LEVEL RADIATION

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THIS series of investigations was commenced in an effort to test conclusions reached by other investigators[1,2] that radiation is a 'noxious stimulus' capable of resulting in avoidance conditioning. In three independent attempts to reproduce 'spatial conditioned avoidance' at this Laboratory, no success was obtained. In each of these experiments, albino rats were on alternate days exposed to 1 r. per min. of X-radiation while confined in a black compartment. During subsequent test periods, the rats were given free access to black and white shuttle-box compartment. No significant avoidance of the black compartment was observed in any of the three experiments when the behaviour of the experimental subjects was compared with that of control subjects.

Although no 'conditioned avoidance' was observed in the initial experiments, it could not be concluded that rats are unable to perceive radiation. A more sensitive procedure would seem to be one that allows rats to choose between two shuttle-box compartments —one located in a radiation field and the other shielded from radiation by a lead screen. Because rats normally prefer the darker of two compartments, it was considered appropriate to use shuttle-boxes with one dark and one light compartment and to place the darker compartment in the exposed area. Dose-rates of 0, 1, 2 and 4 r. per min. were employed in this experiment. The results clearly indicated that the experimental animals spent less than a normal amount of time in the usually preferred darker compartment. It was concluded that the presence of radiation in a normally preferred surrounding results in reduced preference for that surrounding; however, no conclusion concerning the nature of the mechanism responsible for the change was possible. Did the rats perceive the radiation, or its immediate consequences, and associate it with the black compartment? Did physiological changes resulting from the radiation produce stimuli which simply overshadowed the normal preference for the dark compartment?

A new experiment was designed which was like the preceding one except that it involved three groups: (*a*) 'radiation experimental' with exposure in the darker compartment and no exposure in the lighter compartment; (*b*) 'non-radiation control' with no exposure in either compartment, and (*c*) 'radiation control' with exposure in both the lighter and darker

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compartments. The brightness contrast between the two compartments was systematically varied in five steps from black-white to black-black. Both male and female rats were used. It was reasoned that if the previously observed change in shuttle-box behaviour had resulted from the rats learning to discriminate between the exposed and non-exposed areas, then the 'radiation experimental' subjects should again evidence this change, but the 'radiation control' groups should behave no differently from the 'non-radiation controls'. On the other hand, if radiation-produced stimuli simply overshadowed the normal brightness preference, then both of the radiation groups should differ from the 'non-radiation controls' but not from each other. The results clearly favoured the latter alternative, and it was concluded that most of the change in preference for the normally preferred environment was the result of a generalized response to altered physiological state and did not result from the perception of radiation and the learning of an avoidance response. This type of change was significantly more pronounced among female rats. Although the results clearly indicated that the preponderance of the observed effect did not depend upon a perceptual-learning mechanism, the probable presence of such a mechanism was suggested by the results. The two rats in the 'radiation experimental' group which were placed in shuttle-boxes with two otherwise identical black compartments spent an average of only 19 per cent of the time in the exposed compartment. Using a pooled estimate of error derived from the complete results, this value was significantly below chance.

An experiment was designed using only shuttle-boxes with two identical black compartments. Both male and female rats were used and, in an effort to identify the mechanism responsible for the previously noted sex differences, both male and female gonadectomized rats were also included. For half the rats the left-hand compartment was shielded, and for half the right-hand compartment was shielded. The response of normal animals to the two shield positions was significantly different from that of the gonadectomized animals, that is, on the average the normal animals moved in the direction of the shielded compartment while the gonadectomized rats indicated a slight trend in the opposite direction. Actually, all the observed shift among normal animals was attributable to the normal male group, with normal females indicating virtually no change.

A replication of the black-black shuttle-box experiment with 20 additional normal male rats yielded mean differences in the direction of the shielded compartment almost identical with those for normal males in the preceding experiment; however, large variance resulted in no statistically significant results in this later experiment.

A final experiment to be mentioned involved shuttle-boxes with one black and one medium-grey compartment. For half the rats the black side was shielded and for half the grey side was shielded. A statistically significant shift in the direction of the shielded compartment was observed; and the magnitude of the observed shift was greater for male rats, although the sex differences were not statistically significant.

The results of these later experiments in which the only difference between the two treatments is a difference in the location of a protective lead shield strongly suggest that a perceptual mechanism may take a minor but real role in the behavioural response of albino rats to low-level radiation and that this perceptual response is stronger in male rats. This latter conclusion is in contrast to the stronger "non-perceptual" response among female rats which was noted in earlier experiments, and which was incidentally verified in the last mentioned experiment through comparison with a non-radiation control group.

Radiation exposure produces changes in the amount of time that an albino rat will spend in a normally preferred surrounding. Two mechanisms are suggested. The major determinant of the change is a generalized response to a radiation-induced physiological state. A perceptual-learning mechanism may take a minor role in the behavioural change observed in male rats.

The dose-rates employed in all experiments except the one where additional dose-rates are specifically stated was 1 r. per min. in the air inside the shuttle-box compartments.

This work was conducted at the Radiobiological Laboratory of the University of Texas and the United States Air Force, Austin, Texas, and was supported (in part) with funds provided under Contract *AF* 41(657)–149 and the School of Aviation Medicine.

[1] Garcia, J., Kimeldorf, D. J., and Koelling, R. A., *Science*, **122**, 157 (1955).
[2] Garcia, J., Kimeldorf, D. J., and Hunt, B. A., *Brit. J. Radiol.*, **20**, 318 (1957).

ENERGY-LINKED REDUCTION OF MITOCHONDRIAL PYRIDINE NUCLEOTIDE

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IN thermodynamic computations of reactions in biological systems[1] it has been customary to assume that the formation of reduced pyridine nucleotide proceeds in an exergonic reaction in which the free energy of the oxidation-reduction couple ($AH_2 \cdots \rightarrow A$) is sufficiently negative with respect to reduced pyridine nucleotide itself that no external energy source is required to drive the reaction[1]. This article presents experimental results suggesting that the reduction of mitochondrial pyridine nucleotide can be accomplished by oxidation-reduction couples having potentials several hundred millivolts higher than that of the pyridine nucleotide couple[1–3].

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This discovery opens up new possibilities for utilization of substrates of high oxidation-reduction potential for pyridine nucleotide reduction with subsequent oxidation in various energy-yielding or synthetic reactions. It also indicates that the respiratory chain can utilize energy conserved at some sites to drive energy-requiring reactions at others.

The remarkable effect of succinate upon the reduction of intramitochondrial pyridine nucleotide was observed early in studies of rat liver mitochondria[4–6] and has recently been studied by Klingenberg *et al.*[18]. This reaction differs sharply from the fumarate oxidation of reduced pyridine nucleotide in non-phosphorylating Keilin and Hartree particles[7]:

$$\text{Fumarate} + \text{DPNH} + \text{H}^+ = \text{DPN}^+ + \text{succinate} \quad (1)$$

The reverse of this reaction requires an energy expenditure since the reductant couple ($A'\text{H}_2 \cdots\cdots \rightarrow A'$) affords insufficient free energy to reduce pyridine nucleotide:

$$\text{DPN}^+ + A'\text{H}_2 + X \sim I = \text{DPNH} + \text{H}^+ + A' + X + I \quad (2)$$

The driving force for such a reaction could be a high-energy intermediate of oxidative phosphorylation ($X \sim I$), which provides the energy required by the difference of oxidation-reduction potentials between the $A'\text{H}_2 \text{- - -} \rightarrow A'$ couple and the pyridine nucleotide couple. In effect, $X \sim I$ raises the potential of pyridine nucleotide or lowers that of $A'\text{H}_2$ sufficiently to allow the reaction to proceed to completion[8]. This article illustrates the kinetics of reduction of pyridine nucleotide by succinate, particularly in kidney and heart mitochondria, where the extent of reduction of pyridine nucleotide by succinate greatly exceeds that of the usual diphosphopyridine nucleotide-linked substrates. The nature of the substance reduced is identified by spectroscopic and fluorometric data. In addition, the pathway of electron transfer is shown to involve carriers of the respiratory chain, the whole cytochrome chain for oxidation, and part of the chain for reduction. The great sensitivity of the reaction to energy expenditure (for example, by adenosine diphosphate) or to uncoupling of the phosphorylation clearly demonstrates the energy requirements for this reaction. In addition, a direct demonstration of a metabolic recovery process following energy expenditure in pyridine nucleotide reduction has been made.

Material and Methods

Rat liver[9] and guinea pig kidney mitochondria[10], and rat heart sarcosomes[11,12] were prepared by methods described elsewhere. The spectrophotometric[13,14] and polarographic[15] apparatus have been described previously.

Fluorometric measurement of the extent of reduction of mitochondrial pyridine nucleotide[16,17] is used to considerable advantage in rat heart preparations where this component is more difficult to observe than in rat liver preparations. It should be noted that Klingenberg *et al.*[18] (see particularly their Fig. 3*b*) have confirmed the difficulty of making measurements of reduced pyridine nucleotide in rat heart preparations due to the change in absorbancy at 320 mμ[12]; the fluorometric method has much to recommend it for assays of reduced pyridine nucleotide where partial depletion of mitochondrial pyridine nucleotide has occurred and where the chemical method may fail to demonstrate its presence[18].

Succinate-linked Reduction of Pyridine Nucleotide

The data of Figs. 1 *A* and *B* provide examples of spectrophotometric and fluorometric recordings of pyridine nucleotide reduction in kidney and heart mitochondria. The recordings of Fig. 1*A* were obtained with the double-beam spectrophotometer set at measuring wave-lengths appropriate for the reduction of pyridine nucleotide (340 or 350 mμ) and at a nearby reference wave-length (374 mμ). The same phenomenon can be recorded fluorometrically with 366 mμ excitation and 443 mμ measurement[17]. Treatment of the mitochondria in state 1 with adenosine diphosphate gives state 2, a more highly oxidized state of pyridine nucleotide. Addition of 4 m*M* glutamate causes pyridine nucleotide reduction (downward deflexion of the traces) and initiates respiration until the added adenosine diphosphate is exhausted, when a further reduction of pyridine nucleotide is observed (state 4_g). The process may be repeated with a second addition of adenosine diphosphate, as in Fig. 1*B*. In both cases addition of 4 m*M* succinate causes a large, rapid downward deflexion of the trace which these studies indicate to be due to an increased reduction of pyridine nucleotide (state 4_s). Addition of adenosine diphosphate to the mitochondria treated with glutamate and succinate causes an oxidation of the reduced pyridine nucleotide (state 3_s). These experiments demonstrate that the extent of reduction of pyridine nucleotide by succinate considerably exceeds that obtainable with glutamate alone. Mitochondria isolated from ascites tumour cells also show this effect[19]. Bücher and Klingenberg have demonstrated the reduction of pyridine nucleotide in locust sarcosomes by α-glycerophosphate[20,21]. More recently, Klingenberg *et al.*[18] have confirmed our studies on heart and kidney mitochondria. Other pyridine nucleotide-linked substrates of the citric acid cycle have no greater effect than glutamate, and the succinate effect is

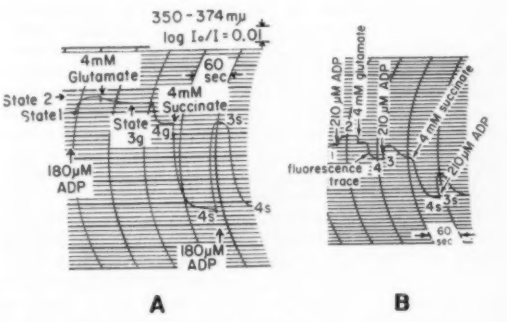


Fig. 1. Comparison of the effects of glutamate and succinate on pyridine nucleotide reduction in guinea pig kidney (*A*) and rat heart mitochondria (*B*). The time-scale increases from left to right; the reagents and states of the mitochondria are indicated in the figures. *A*, spectrophotometric recording at 350–374 mμ; *B*, fluorometric recording, 366 mμ excitation, 443 mμ measurement. Mitochondria suspended in isotonic medium, 26°. (Expts. 658*a*–10, 791–1)

sensitive to malonate (50 per cent inhibition for malonate/succinate = 0·35).

The pathway of the succinate-linked pyridine nucleotide reduction is not the main pathway of electron transfer; the maximal rate of reduction is about equal to the state-4 rate of oxygen utilization or roughly one-fifth the state-3 rate. Nevertheless, this pathway appears to have considerable theoretical interest and may be of physiological importance.

It is important to clarify the results obtained previously with rat liver mitochondria[22]. In this material, succinate causes reduction of pyridine nucleotide, but its effect is not appreciably in excess of that caused by β-hydroxybutyrate. In more recent comparisons of the effects of glutamate and succinate on rat liver mitochondria, similar to those of Fig. 1, the ratio of the increment on adding succinate (4_s–4_g) to the effect of glutamate alone (4_g–2) is only 0·22. The corresponding average values for heart and kidney mitochondria are 2·1 and 3·3 respectively. The discovery of a large amount of triphosphopyridine nucleotide in mitochondria[20,23] affords an explanation for this difference: apparently triphosphopyridine nucleotide is affected similarly by succinate and β-hydroxybutyrate and thereby obscures accurate spectrophotometric detection of differences in the effect of these two substrates upon the diphosphopyridine nucleotide of liver. For this reason, kidney and heart mitochondria are more suitable for spectrophotometric and fluorometric studies than are liver mitochondria. In previous studies we noted a much smaller pyridine nucleotide–cytochrome *a* ratio in rat heart than in rat liver mitochondria when using α-oxoglutarate as substrate[13]. The larger pyridine nucleotide reduction obtainable with succinate now gives a ratio of ~ 9 : 1, which is about half that for liver mitochondria[24].

Nature of Reduction Product

Spectrophotometric and fluorometric data. To identify the material reduced by succinate and oxidized by adenosine diphosphate, as indicated in Fig. 1, we have recorded spectra representing absorbancy differences between state 4_s and state 3_s in succinate-reduced mitochondria. Fig. 2 also represents studies of two preparations, one from guinea pig kidney (*A*) and one from rat heart (*B*). The increased absorbancy on adding succinate to substrate-free mitochondria (state 2) to give 340 mμ absorption (state 4) is indicated in Fig. 2*A*. Fig. 2*B* shows that the addition of adenosine diphosphate to succinate-treated mitochondria (state 4) causes a band with a maximum at 340 mμ to disappear (note the negative values of the ordinate). The peaks of the spectra are close to 340 mμ, as would be expected for the oxidation and reduction of pyridine nucleotide. Other studies show the succinate-reduced material to have a fluorescence maximum at 443 mμ[16].

It appears that the succinate-reduced material has the physical properties characteristic of the reduced state of pyridine nucleotide. Since, as indicated below, the material so reduced can be oxidized in an amytal-sensitive reaction by the respiratory chain, it is concluded that a reduced form of pyridine nucleotide is formed in this reaction. It is further concluded that the reduced form is bound to some ligand, for example, a protein, because the fluorescence maximum is at 443 mμ[25,26]. Thus this material is denoted DPNH ~ *I*[27] although Slater currently uses the description DPN ~ *I*H (cf. refs. 29, 35). Actually, the two forms could resonate. There are no data available to indicate the chemical nature of ~ *I*. We found that DPNH ~ *I* was refractory to acetoacetate[30,31] or to ferricyanide[32] oxidation, and Purvis believes that it is refractory to low concentrations of alcohol dehydrogenase[33] (but compare below and ref. 34). The interesting possibility that ubiquinone–coenzyme *Q* may be the ligand[35] remains an unsupported hypothesis and now appears to be inconsistent with the reduced pyridine nucleotide and ubiquinone contents of guinea pig kidney mitochondria, which are in the ratio of about 4 : 1 (ref. 36).

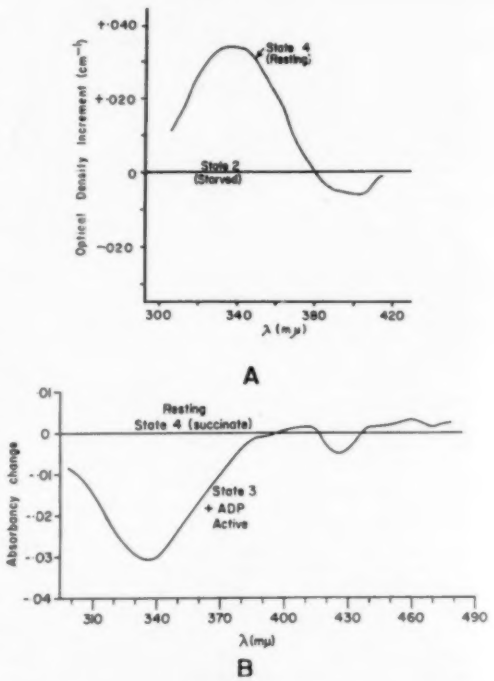


Fig. 2. Spectroscopic changes caused by succinate addition to 'starved' guinea pig kidney mitochondria (*A*) and adenosine diphosphate addition to succinate-treated rat heart mitochondria (*B*). Conditions similar to Fig. 1. (Expts. 664*b*–1, 771*c*–3)

Chemical data. Chemical determinations of the material reduced by succinate, particularly in the kidney mitochondria, are in agreement with the conclusion above[16,27] (also Bücher and Klingenberg, personal communication). The increased absorbancy obtained on adding succinate to the kidney mitochondria is very nearly equal to the decrease of absorbancy caused by adding alcohol dehydrogenase and acetaldehyde to a neutralized sodium carbonate extract of such mitochondria. Thus, it can be said that nearly the same amount of material reduced by succinate in the guinea pig kidney mitochondria can be re-oxidized by dilute yeast alcohol dehydrogenase and acetaldehyde in an extract. This result differs from that obtained by Purvis[33] and Slater[28], who used rat liver mitochondria. It also indicates that diphosphopyridine nucleotide is involved, in agreement with Bücher and Klingenberg[13] (and personal communication). Thus, succinate acts as a powerful

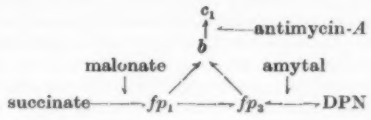
reductant of mitochondrial diphosphopyridine nucleotide.

Pathway of Reduction and Oxidation

It is now appropriate to consider the pathway for reduction and oxidation of mitochondrial pyridine nucleotide by succinate. The fact that the oxidation-reduction of succinate-reduced diphosphopyridine nucleotide closely follows the increases and decreases of respiratory activity accompanying the state 4–3–4 transitions suggests that this component is closely associated with the respiratory chain. The oxidation of reduced diphosphopyridine nucleotide has been found to be sensitive to cyanide, antimycin-*A*, and amytal. It can therefore be concluded that pyridine nucleotide reduced by succinate is oxidized by the same pathway as the pyridine nucleotide reduced by the usual diphosphopyridine nucleotide-linked substrates.

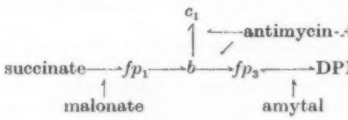
Pyridine nucleotide reduction could be activated by a decrease in the velocity of its oxidation or by an increase in the velocity of its reduction, either along the usual pathway or by the activation of a new pathway. We have carefully examined the possibility that the addition of succinate decreases the flow of oxidizing equivalents to reduced pyridine nucleotide by reducing the steady-state oxidation-level of cytochrome *b*. In this case, addition of excess cyanide should cause pyridine nucleotide reduction at a rate exceeding that caused by addition of succinate. This has not been observed in freshly prepared mitochondria with either cyanide or hydrosulphide as inhibitor. In mitochondria depleted of endogenous substrate, addition of cyanide after glutamate causes a slow and incomplete reduction of pyridine nucleotide, whereas addition of succinate causes a complete and three times as rapid reduction. These experiments appear to eliminate a decrease of reduced pyridine nucleotide oxidase activity as a complete explanation. Thus we have considered the succinate effect to be an activation of pyridine nucleotide reduction. Since the increased rate of pyridine nucleotide reduction by succinate is slowed by amytal (see below), we can eliminate an increase of glutamic dehydrogenase activity. The succinate-linked effect probably results from an activation of pyridine nucleotide reduction through an amytal-sensitive flavoprotein interaction, essentially a reversal of electron transfer between cytochrome *b* and reduced pyridine nucleotide via flavoprotein (cf. ref. 18). The reducing equivalents may flow from succinate directly or from glutamate via a small amount of directly reduced pyridine nucleotide[39] to the larger pool of pyridine nucleotide. The above-mentioned results with cyanide and the unpublished results of Klingenberg and Bücher (cf. ref. 18) on α-glycerophosphate support the former view which is embodied in the equations which follow. Ubiquinone–coenzyme *Q* is not yet included in these equations, but experiments on non-phosphorylating preparations would place it next to flavoprotein[40], in which case the conclusions reached here would be unchanged since direct transfer through ubiquinone, as through flavoprotein, would be insensitive to antimycin *A*.

The pathway for the reduction of the material is also indicated by its inhibitor sensitivity. The malonate and amytal sensitivity of the pathway of reduction of pyridine nucleotide which is demonstrated by spectroscopic experiments suggests a transfer pathway similar to that of equation 3, where interaction between flavoproteins (fp_1 and fp_3) causes the reduction of pyridine nucleotide:



(3)

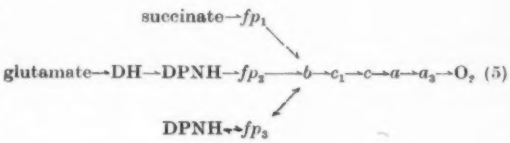
Such a pathway is in accord with the suggestions of Beinert[41], who favours the idea of direct transfer between flavoproteins. The reduction of diphosphopyridine nucleotide by a diaphorase preparation has recently been studied by Massey[42] and by Searls and Sanadi[43]. However, the tenfold increase in the speed of reduction of cytochrome *b* in the amytal-treated material, together with the fact that the reduction of pyridine nucleotide is slower in the presence of antimycin-*A* than in the presence of cyanide, suggests that reducing equivalents for diphosphopyridine nucleotide pass through the respiratory chain at the level of cytochrome *b*:



(4)

Since the site of action of antimycin-*A* is between cytochromes *b* and c_1, it is suggested that an inhibition of the ability of cytochrome *b* to transfer reducing equivalents along the chain has occurred. In the case of pyridine nucleotide reduction, antimycin-*A* would be inhibiting the ability of cytochrome *b* to transfer reducing equivalents to the flavoprotein, fp_3, involved in this reduction reaction (see equation 4). The pathways indicated by this equation are in satisfactory accord with the kinetic data on the effect of the indicated inhibitors upon the kinetics of cytochrome *b*, flavoprotein, and pyridine nucleotide, and represent a working hypothesis for discussions of this reaction.

These pathways of equation 4 are written in the usual abbreviated symbolism for the respiratory chain in equation 5:



(5)

This scheme indicates that the pathway for the oxidation of succinate-reduced diphosphopyridine nucleotide proceeds by the same pathway as the reduction. However, this reduced diphosphopyridine nucleotide could equally well diffuse to another set of respiratory carriers and be oxidized there.

Comparison of Succinate and Glutamate Reduction

The chief result of the experiments so far is to show that a larger reduction of diphosphopyridine

nucleotide occurs by a succinate-linked pathway through the respiratory carriers than can be achieved by diphosphopyridine nucleotide-linked dehydrogenases. A simple kinetic explanation based upon a greater speed of reduction by succinate appears to be inadequate: the glutamate reduction of diphosphopyridine nucleotide in state 3, as indicated by the respiratory activity, exceeds the rate at which succinate can activate pyridine nucleotide reduction, as illustrated in Fig. 1; fp_3 is rapidly reduced but cannot transfer its electrons rapidly to fp_3 and pyridine nucleotide without the mediation of succinate. Thus an adequate kinetic explanation depends upon a greater rate of electron transfer through fp_3 in the presence of succinate than in the presence of glutamate alone. Also, explanation for the partial reduction of the pyridine nucleotide linked to fp_3 by glutamic dehydrogenase activity is required.

An explanation suggesting that diphosphopyridine nucleotide is reduced by the diphosphopyridine nucleotide-linked dehydrogenases of the mitochondria and triphosphopyridine nucleotide by the succinate-linked pathway has no foundation in the chemical analyses of the kidney mitochondria, which show the presence of very little triphosphopyridine nucleotide[18].

A hypothesis based on chemical difference between the glutamate- and succinate-reduced diphosphopyridine nucleotide in mitochondria which are linked to their reductase system is indicated in the equations below:

$$\text{Glutamate} \longrightarrow \text{DH} \longrightarrow \text{DPNH} \sim I \quad (6)$$

$$\text{Succinate} \longrightarrow (\text{Carriers}) \longrightarrow fp_2 \longrightarrow \text{DPNH} \sim I' \quad (7)$$

We here distinguish between the kinds of pyridine nucleotide by the nature of the ligand which inhibits electron transfer in state 4, that appropriate to the glutamic dehydrogenase pyridine nucleotide being designated I and that appropriate to the succinate-linked component being designated as I'. Thus, glutamate has direct access to DPNI and can reduce it directly. Succinate, acting through the respiratory carriers, activates DPNI' through its specific flavoprotein (fp_2). This mechanism explains the way in which succinate and glutamate can reduce different amounts of pyridine nucleotide on the basis of chemical specificity. There are two observations that require an elaboration of this hypothesis. In anaerobiosis, the full amount of diphosphopyridine nucleotide is slowly reduced in the absence of added succinate. Also succinate reduces the total diphosphopyridine nucleotide of the kidney mitochondria in the absence of added glutamate. Both these effects could be explained by diphosphopyridine nucleotide-reduced diphosphopyridine nucleotide transhydrogenase activity[44], which is insufficient to be active aerobically but which nevertheless acts on the two forms of diphosphopyridine nucleotide of equation 6, above, in anaerobiosis.

A slow glutamate-linked transhydrogenase activity would afford a better basis for a kinetic explanation of the different degrees of diphosphopyridine nucleotide reduction by glutamate and succinate. Such an activity could well utilize the pathways of equation 5, fp_1 being more effective than fp_2 in the reduction of pyridine nucleotide via fp_3.

An alternative hypothesis is that diphosphopyridine nucleotide is specifically bound to glutamate- and succinate-linked reducing sites. Such a condition would immediately satisfy the larger reduction of pyridine nucleotide by succinate and a smaller reduction by the usual glutamate-linked and other substrates. Evidence for the binding of mitochondrial pyridine nucleotide has already been presented above from fluorometric experiments. This apparently simple hypothesis becomes more complicated when it is considered that all diphosphopyridine nucleotide-linked substrates apparently reduce the same amount of pyridine nucleotide (α-oxoglutarate, glutamate, malate, β-hydroxybutyrate). Thus, the pyridine nucleotide bound at the glutamate site must be readily available to the other diphosphopyridine nucleotide-linked dehydrogenases (but not to the succinate-linked pathway). Preliminary evidence that adenosine diphosphate (and uncoupling agents) may inhibit the binding of pyridine nucleotide to its succinate-linked site is afforded by fluorometric data which show diminished fluorescence of pyridine nucleotide in anaerobiosis where adenosine diphosphate can accumulate.

There is apparently no difficulty in providing adequate binding sites for pyridine nucleotide at the succinate-linked site, since it is not unusual to have four molecules of pyridine nucleotide bound to a single protein[45].

Compartmentation of an identical chemical species of pyridine nucleotide would also explain the experimental data; it is conceivable that the glutamate-linked material is bound to the cristæ and the succinate-linked material to the matrices. It is necessary to assume that such compartments are slowly equilibrated in anaerobiosis, and that the matrix material can reach fp_3 but not glutamic dehydrogenase in aerobiosis.

Of these hypotheses only the simple kinetic explanation appears inconsistent with available experimental data. Those hypotheses involving: (1) a slow glutamate-linked transhydrogenase activity; (2) chemical specificity; (3) bound pyridine nucleotide; and (4) structural compartmentation must remain under active consideration.

Energy Expenditure in Reduction of Diphosphopyridine Nucleotide

The data above suggest that pyridine nucleotide reduction is associated with not only the reducing power of succinate but also the electron transfer from succinate to oxygen. In fact, the maximal rate of succinate-linked pyridine nucleotide reduction is about equal to the rate of electron transfer in state 4. This correlation, combined with the high sensitivity of the reaction to uncoupling agents, has led us to investigate whether there is a demonstrable energy requirement for this reaction. First, we shall consider the thermodynamic properties of such an energy-linked reaction.

Thermodynamic potential. In considering the energy requirements for the succinate-linked reaction, it is highly desirable to indicate the thermodynamic potential against which this reaction can occur. The graph of Fig. 3 indicates a titration of diphosphopyridine nucleotide of kidney mitochondria with succinate in the absence of fumarate and in the presence of 30 mM fumarate. In the absence of added fumarate, 190 μM succinate gives half-maximal reduction. To obtain an estimate of the potential

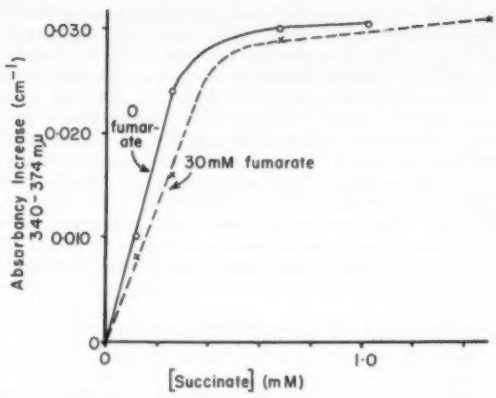


Fig. 3. A titration of pyridine nucleotide of guinea pig kidney mitochondria with succinate in the absence and in the presence of 30 m*M* fumarate. Mitochondria pretreated with 4 m*M* glutamate and then titrated with succinate (cf. Fig. 1). (Expt. 715*d*)

against which this reaction will occur, the titration is repeated in the presence of 30 m*M* fumarate; in this case, 215 μ*M* succinate gives half-maximal reduction. This increase in succinate concentration is so small that the question may be raised as to whether the system is in equilibrium (the fumarate/succinate ratio differs by roughly 10^4 in the two titrations (see also refs. 29, 46)). If we assume an equilibrium, the redox span ranges from 34 mV. (ref. 2) to − 320 mV. (ref. 3) or a total of 354 mV. Even if the assumed equilibrium is proved incorrect, it is clear that the reaction has a considerable energy requirement.

Effect of uncoupling agents. If the overall process involves a high-energy intermediate,

$$\text{Succinate} + \text{DPN}^+ + X \sim I \rightleftharpoons \text{fumarate} + \text{DPNH} + X + I + \text{H}^+ \quad (8)$$

uncoupling agents should inhibit the reaction. Table 1 summarizes the concentration of uncoupling agents giving half-maximal effects. It is seen that extremely low concentrations are effective in inhibiting this reaction. More detailed studies identify an inhibition of the reduction as opposed to an activation of the oxidation because of the effects of the uncoupling agent.

It can be seen that the concentration of mitochondrial reduced pyridine nucleotide can be regulated over wide ranges by balancing the reductive effect of succinate against the oxidizing effect of adenosine diphosphate or of substances such as thyroxine which act at such low concentrations that the phosphorylation efficiency would be largely unaffected.

Effect of adenosine triphosphate. In view of the postulated requirement of the succinate-linked reduction for a high-energy intermediate, it appeared reasonable that the addition of adenosine triphosphate would accelerate the reduction reaction. Mitochondria in which the extent of reduction was inhibited ~ 50 per cent by 24 μ*M* dibromophenol were treated with a high concentration of adenosine triphosphate (0·8 m*M*), but no measurable increase of reduction was observed. Apparently the added adenosine triphosphate caused no increase in the concentration of intermediates suitable for reducing the pyridine nucleotide.

Effect of phosphate. In an effort to determine whether the reduction reaction is driven by an $X \sim I$ or an $X \sim P$ compound, the succinate-linked reduction was demonstrated in a phosphate-free medium and found to proceed unchanged in rate or extent. Control experiments based on the lack of a spectroscopic response to the addition of adenosine diphosphate suggested that less than one-twelfth the required amount of $X \sim P$ (see calculations below) could have been present under the experimental conditions.

Recovery from energy expenditure. Equation 8 suggests that the breakdown of $X \sim I$ would result in an activation of oxidative recovery to restore the energy expended in reduction of diphosphopyridine nucleotide. If mitochondria are treated with a low concentration of azide (50 μ*M*), changes in the steady state oxidation-reduction level of the cytochromes due to activation of electron transfer in oxidative recovery are sensitively indicated[47] (Fig. 4). Mitochondria pretreated with glutamate and azide are in state 4. Addition of succinate causes a downward deflexion of the fluorometric trace which corresponds to increasing reduction of pyridine nucleotide. Simultaneously, the trace corresponding to cytochrome *a* shows a cycle of reduction and oxidation which suggests that oxidative recovery is initiated upon adding succinate.

The experiment of Fig. 4 can be made quantitative if a known amount of adenosine diphosphate is added to compare its effect with that of the succinate. Thus, 110 μ*M* adenosine diphosphate is added, giving a larger and longer cycle of reduction of cytochrome *a* than did succinate. (The fluorescence trace shows that, in this instance, reduced pyridine nucleotide

Table 1. Concentrations of Uncoupling Agents* giving 50 per cent Inhibition of the Rate of DPN Reduction by 4 m*M* Succinate (K_u). Guinea Pig Kidney Mitochondria, Sucrose-Salt Medium, 26°

Uncoupling agent	DBP	DNP	DiC	DiB	Thyroxine	
Experiment number	722	722	723*b*	723*d*	719*b*	721*b*
Mg^{++}	5 m*M*	5 m*M*	5 m*M*	5 m*M*	5 m*M*	—
Initial state	4 *g*	4 *g*	4 *g*	4 *g*	1	1
Substrate	4 m*M* glut-amate	4 m*M* glut-amate	4 m*M* glut-amate	4 m*M* glut-amate	—	—
'K_u' (μ*M*)	22	3·0	1·2	1·0	3·2	0·5

* DBP, Dibromophenol; DNP, Dinitrophenol; DiC, Dicoumarol; DiB, Butyl-3,5-diiodo-4-hydroxybenzoate.

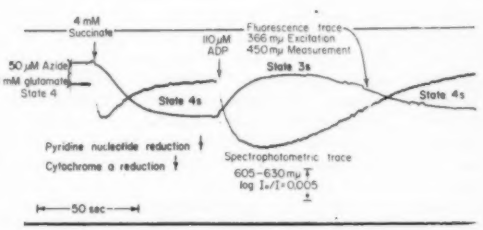


Fig. 4. A demonstration of recovery from energy expended in the reduction of pyridine nucleotide by succinate. Rat liver mitochondria pretreated with 4 m*M* glutamate and 50 μ*M* azide. Energy recovery is indicated by the cycle of reduction and oxidation of cytochrome *a* (measured at 605–630 mμ) caused by the succinate-linked reduction of pyridine nucleotide. 110 μ*M* adenosine diphosphate is added to calibrate the energy recovery (10°). (Expt. 9706–13)

is oxidized, as is the usual case in the state 4-to-3 transition.) A comparison of the areas under the curves corresponding to addition of adenosine diphosphate and succinate reveals that 8 per cent of the area corresponding to 110 μM adenosine diphosphate is caused by succinate addition. In other words, a resynthesis of a high-energy intermediate corresponding to the utilization of 9 μM adenosine diphosphate (22·5 mμmoles) is required. If the amount of mitochondria is varied, a straight-line relationship between the amount of mitochondrial protein and the amount of respiratory resynthesis is found, suggesting that a stoichiometric relationship exists between energy expenditure and pyridine nucleotide reduction. Finally, computing, by suitable calibrations based upon absorbancy changes at 340 mμ, the number of adenosine diphosphate equivalents required to form 1 mole of reduced diphosphopyridine nucleotide, a ratio of $\sim 3:1$ is obtained. It is further found that pretreatment of the mitochondria with amytal does not reduce the energy expenditure upon succinate addition to zero; 30 per cent remains. Thus, for the specific reduction of diphosphopyridine nucleotide by succinate, 2 adenosine diphosphate equivalents are required. We may make preliminary assignments of the energy expenditure at the sites indicated by the following equation:

$$\begin{array}{l}\text{Succinate} \rightarrow fp_1 \rightarrow b \rightarrow fp_2 \rightarrow \text{DPNH} \rightarrow \text{DPNH} \sim I \\ \qquad\qquad\quad \uparrow \qquad\quad \uparrow \qquad\quad \uparrow \\ \qquad\qquad\quad X \sim I \quad X \sim I \quad X \sim I\end{array} \tag{9}$$

This suggests that the succinate-linked reaction not only reduces pyridine nucleotide to the level of − 320 mV. but, further, forms a high-energy bond between it and its ligand, I. However, the technique used in the assay of the adenosine diphosphate equivalents formed is a novel one and some confirmation by independent means would appear to be desirable.

It is not surprising that the energy expenditure in this reduction is high relative to the one adenosine triphosphate equivalent obtained in the oxidation of reduced diphosphopyridine nucleotide because the reduction reaction must pay the full cost of the reaction. For the span between fp_2 and DPNH $\sim I$, the energy conserved as adenosine triphosphate in the oxidation reaction appears to be about one-half of the energy cost of reduction. In this sense, this experiment gives us a rough estimate of the efficiency of oxidative phosphorylation in this part of the chain.

'*Energy transferase*'. It is apparent that high-energy intermediates formed in the cytochrome portion of the chain by succinate oxidation can be used to reduce diphosphopyridine nucleotide. It is also true that glutamate treatment of the mitochondria increases the speed of the succinate-linked reduction, suggesting that the high-energy intermediate formed from diphosphopyridine nucleotide can also participate in diphosphopyridine nucleotide reduction. This is a sort of transhydrogenase activity. In addition, such reduced diphosphopyridine nucleotide can, by its oxidation, form other high-energy intermediates. Thus, there can be an equilibration of high-energy intermediates in the respiratory chain, essentially an 'energy transferase' activity as suggested some time ago by Lipmann[48]. The existence of such a reaction further suggests that intermediates of various energy contents may be used to increase the ratio of reduced to oxidized diphosphopyridine nucleotide until the potential of the system is sufficient to form an intermediate capable of phosphorylating adenosine triphosphate. Such a possibility greatly widens the range of sites that can be considered for energy conservation and may ultimately make the assignment of a particular phosphate bond to a particular redox couple unrealistic. Thus, even more attention may be focused upon the experimentally observed loci, namely, the three sites at which inhibitory interactions are identified by the crossover theorem[49].

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[1] Burton, K., and Krebs, H. A., *Biochem. J.*, **54**, 94 (1953).
[2] Borsook, H., and Schott, H. F., *J. Biol. Chem.*, **92**, 535 (1931).
[3] Burton, K., and Wilson, T. H., *Biochem. J.*, **54**, 86 (1953).
[4] Chance, B., and Williams, G. R., *Fed. Proc.*, **14**, 190 (1955).
[5] Chance, B., and Hollunger, G., *Fed. Proc.*, **16**, 163 (1957).
[6] Chance, B., in "Enzymes: Units of Biological Structure and Function", 447, ed. by Gaebler, O. H. (Academic Press, Inc., New York, 1956).
[7] Slater, E. C., *Biochem. J.*, **46**, 484 (1950).
[8] Glahn, P. E., and Nielsen, S. O., *Nature*, **183**, 1578 (1959).
[9] Lardy, H. A., and Wellman, H., *J. Biol. Chem.*, **195**, 215 (1952).
[10] Hollunger, G., *Acta Pharmacol. et Toxicol., Scand.*, **11**, Suppl. 1 (1955).
[11] Cleland, K. W., and Slater, E. C., *Biochem. J.*, **53**, 547 (1953).
[12] Chance, B., and Baltscheffsky, M., *Biochem. J.*, **63**, 283 (1958).
[13] Chance, B., *Science*, **120**, 767 (1954).
[14] Yang, C. C., and Legallais, V., *Rev. Sci. Instr.*, **25**, 801 (1954).
[15] Chance, B., and Williams, G. R., *J. Biol. Chem.*, **217**, 383 (1955).
[16] Chance, B., and Baltscheffsky, H., *J. Biol. Chem.*, **233**, 736 (1958).
[17] Chance, B., Conrad, H., and Legallais, V., Programme and Abstracts, Biophysical Society, Cambridge, Massachusetts, 44 (1958).
[18] Klingenberg, M., Slenczka, W., and Ritt, E., *Biochem. Z.*, **332**, 47 (1959).
[19] Chance, B., and Hess, B., *J. Biol. Chem.*, **235**, 2413 (1959).
[20] Bücher, Th., and Klingenberg, M., *Angew. Chemie*, **70**, 552 (1958).
[21] Klingenberg, M., and Bücher, Th., *Biochem. Z.*, **331**, 312 (1959).
[22] Chance, B., and Williams, G. R., *J. Biol. Chem.*, **217**, 409 (1955).
[23] Jacobson, K. B., and Kaplan, N. O., *J. Biol. Chem.*, **226**, 603 (1957).
[24] Chance, B., and Williams, G. R., *J. Biol. Chem.*, **217**, 395 (1955).
[25] Boyer, P. D., and Theorell, H., *Acta Chem. Scand.*, **10**, 447 (1956).
[26] Duysens, L. N. M., and Kronenberg, G. H. M., *Biochim. et Biophys. Acta*, **26**, 437 (1957).
[27] Chance, B., Williams, G. R., Holmes, W., and Higgins, J., *J. Biol. Chem.*, **217**, 439 (1955).
[28] Slater, E. C., in Ciba Foundation Symposium on Cell Metabolism, ed. by Wolstenholme, G. E. W., and O'Connor, C. M. (J. and A. Churchill, Ltd., London, 1959).
[29] Slater, E. C., in Proceedings of the Hæmatin Enzyme Symposium, Canberra, Australia, 1959 (in the press).
[30] Chance, B., and Williams, G. R., "Advances in Enzymol.", **17**, 65 (1956).
[31] Chance, B., in Ciba Foundation Symposium on Cell Metabolism, ed. by Wolstenholme, G. E. W., and O'Connor, C. M. (J. and A. Churchill, Ltd., London, 1959).
[32] Estabrook, R. W., and Mackler, B., *Fed. Proc.*, **15**, 248 (1956).
[33] Purvis, J. L., Abst., Amer. Chem. Soc., p. 5*C* (Boston, April 1959).
[34] Estabrook, R. W., in Proceedings of the Hæmatin Enzyme Symposium, Canberra, Australia, 1959 (in the press).
[35] Slater, E. C., Spallanzani Lecture, 1959.
[36] Chance, B., *Fed. Proc.* (in the press).
[37] Chance, B., and Hollunger, G. (in preparation).
[38] Purvis, J. L., *Nature*, **182**, 711 (1958).
[39] Devlin, T., *J. Biol. Chem.*, **234**, 962 (1959).
[40] Chance, B., and Redfearn, E. R., Abst., Amer. Chem. Soc., Cleveland (April 1960).
[41] Beinert, H., and Crane, F. L., *J. Biol. Chem.*, **218**, 717 (1955).
[42] Massey, V., *Biochim. et Biophys. Acta*, **30**, 205 (1958).
[43] Searls, R. L., and Sanadi, D. R., *Proc. U.S. Nat. Acad. Sci.*, **45**, 697 (1959).
[44] Stein, A. M., Kaplan, N. O., and Ciotti, M. M., *J. Biol. Chem.*, 234-979 (1959).
[45] Velick, S. F., in "Mechanism of Enzyme Action", 499, ed. by McElroy, W. D., and Glass, B. (Johns Hopkins Press, Baltimore, 1954).
[46] Chance, B., and Hollunger, G. (in preparation).
[47] Chance, B., and Williams, G. R., *J. Biol. Chem.*, **221**, 477 (1956).
[48] Lipmann, F., in "Currents in Biochemical Research", 137, ed. by Green, D. E. (Interscience Publishers, Inc., New York, 1956).
[49] Chance, B., Holmes, W., Higgins, J., and Connelly, C. M., *Nature*, **182**, 1190 (1958).

LETTERS TO THE EDITORS

VIROLOGY

Sub-Units in Southern Bean Mosaic Virus

Two results have emerged from preliminary X-ray diffraction studies of single crystals of southern bean mosaic virus. The first, and the more important one, is information about the structure of the virus particle proper, and the second about the packing of the particles in the crystal. Each particle is composed of identical sub-units, and the particles are arranged in hexagonal close-packing to form the crystal. The fact that this virus, like other small 'spherical' ones previously studied, is composed of sub-units again confirms the hypothesis made by Crick and Watson[1]. They suggested that the protein sub-units pack in cubic point-group symmetry and that there are either 12, 24 or 60 such units depending on whether the symmetry of the particle is tetrahedral, octahedral or icosahedral.

Southern bean mosaic virus crystallizes in the orthorhombic system with a probable space group of $C222_1$. There are four particles in the unit cell of dimensions $a = 295$ Å., $b = 508$ Å. and $c = 474$ Å. Because the ratios of cell dimensions are $b/a = \sqrt{3}$ and $c/a = 1{\cdot}61$, and all interparticle distances are probably equal, the virus particles must be hexagonally close-packed in the crystal. Thus, the centre-to-centre distances of the particles in the crystal are 295 Å. This diameter of virus is essentially the same as the value of 286 Å. obtained from small-angle scatterings of X-rays by southern bean mosaic virus particles in suspension[2].

Diffraction patterns of the wet crystals grown in distilled water show reflexions down to spacings of 5 Å., indicating relatively high crystal perfection, with one virus particle very similar if not identical to another. Two of the patterns, $hk0$ and $0kl$, are shown in Figs. 1*a* and *b*. The conspicuous features of these patterns are the regions of high intensity which correspond to the transform of the individual particle. Although the lattice imposes 222 symmetry on the particle, the positions of the high-intensity streaks (rotation axes marked in Fig. 1) are those of the 532 axes of the cubic icosahedral point group. The three perpendicular two-fold axes are parallel to the *a*-, *b*- and *c*-axes of the orthorhombic lattice.

If the virus has true icosahedral symmetry, it must be composed of sixty identical or nearly identical protein sub-units. Should the sub-units differ by one or two amino-acids, this would not be detectable in the X-ray patterns. Each sub-unit would have a molecular weight of 85,000, as calculated from the 21 per cent nucleic acid[3] and the molecular weight of the virus of $6{\cdot}63 \times 10^6$ (ref. 4). However, these units may be sub-divided. From amino-acid analysis it is known that the protein chain has a minimum molecular weight of 27,000 (ref. 5). Thus, there are probably three similar protein chains in each of the sixty equivalent units of molecular weight 85,000. The ribonucleic acid, by analogy with the other viruses and from preliminary small-angle scattering of X-rays, is probably located within the protein framework. Little is known about it from the X-ray observations at this stage of investigation, except that if it is not a randomly oriented chain it must have at least four sub-units subject to 222 symmetry. That there may be four sub-units merely means that these units are similar structurally but not chemically.

Southern bean mosaic virus resembles tomato bushy stunt[6], turnip yellow mosaic[7], and polio[8] in that all these particles are probably composed of sixty identical or near-identical protein sub-units which pack in icosahedral symmetry to form the framework of the virus. However, at present, neither the shape of the protein sub-units nor that of the virus itself is known about any of the particles examined. By comparing the data for all these particles, it can be seen that southern bean mosaic virus differs from the other three. The diffraction patterns have a different aspect since the streaks of high intensity are much broader for southern bean mosaic virus. It is not unlikely that the shape of the sub-unit affects the sharpness of the streaks.

Another dissimilarity is in the number of interparticle contact points in the lattice. The other plant viruses crystallize in the cubic system, giving body-centred packing with the contact points of the particles along the three-fold axis of both the cell and the particle. Polio virus, although it crystallizes in the orthorhombic class, packs in a lattice which is scarcely distinguishable from body-centred cubic. In the case of southern bean mosaic virus, due to the hexagonal close packing there are four different contact points: two in the hexagonal plane, one of which is along one of the two-

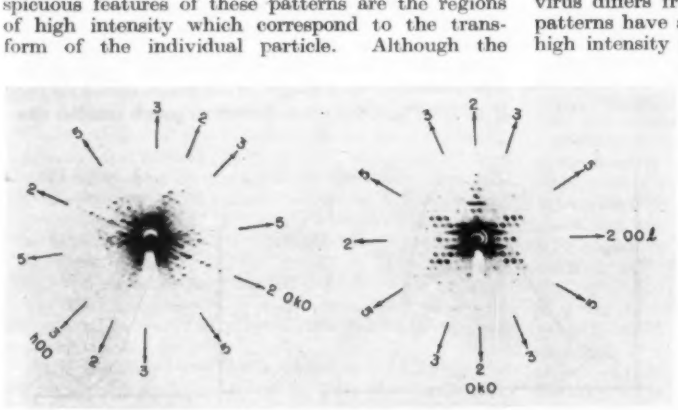

Fig. 1. 2° precession photographs of southern bean mosaic virus crystals taken with the X-ray beam parallel to (*a*) the *c*-axis and (*b*) the *a*-axis. The orientations of the five-, three- and two-fold rotation axes of the icosahedral point group 532 are indicated and coincide with the directions of the strongest intensities

fold axes of the cell, and the other along the cell diagonal, close to a five-fold axis of the particle. The contact points of one particle to its three neighbours in the next layer are of two kinds and differ from those in the plane.

More detailed results will be published in the *Contributions from Boyce Thompson Institute*.

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[1] Crick, F. H. C., and Watson, J. D., *Nature*, **177**, 473 (1956); "The Nature of Viruses", Ciba Foundation Symposium (J. and A. Churchill, London, 1957).
[2] Leonard, jun., B. R., Anderegg, J. W., Shulman, S., Kaesberg, Paul, and Beeman, W. W., *Biochim. Biophys. Acta*, **12**, 499 (1953).
[3] Miller, Gail Lorenz, and Price, W. C., *Arch. Biochem.*, **11**, 337 (1946).
[4] Miller, Gail Lorenz, and Price, W. C., *Arch. Biochem.*, **10**, 467 (1946).
[5] Magdoff, Beatrice S., Block, Richard J., and Montie, Diane Block, *Contrib. Boyce Thompson Inst.*, **18**, 371 (1956).
[6] Caspar, D. L. D., *Nature*, **177**, 475 (1956).
[7] Klug, A., Finch, J. T., and Franklin, R. E., *Nature*, **179**, 683 (1957); *Biochim. Biophys. Acta*, **25**, 242 (1957).
[8] Finch, J. T., and Klug, A., *Nature*, **183**, 1709 (1959).

PHYSICS

Pumping Effect of a Gas Discharge High-Vacuum Pump

Six years ago Foster *et al.*, of the California Radiation Laboratory, constructed a high-vacuum pump with high pumping speed (3,000–7,000 litres/sec.) based on the principle of transport of ions during a discharge in a magnetic field[1]. The limiting pressure reached was less than 1×10^{-6} mm. mercury and the residual gases were free of hydrocarbon vapour. This pump at first caused much interest, but was soon forgotten in favour of more economic pumps based on the pumping effect of evaporated titanium[2].

In our laboratory we have studied some properties of this pump[3]. In the first place, it was necessary to construct a gas discharge pump for experimental work on a small scale, so that it would be relatively easy to make constructional alterations. The discharge tube was made of hard glass (diameter 42 mm., length 1,200 mm.); the inlet opening was at the centre of the tube, the two outlet openings being at the respective ends and connected to a rotary pump by a two-way stop-cock. At both ends an electrode system was point-welded to molybdenum seals. Each electrode system consists of a cathode (a heated tungsten filament and molybdenum target) and a molybdenum ring anode fixed to two long molybdenum tie-beams enclosed in glass. In direct proximity to the anodes in the direction towards the centre, the tube was narrowed for a length of about 5 cm. to a diameter only a little larger than the diameters of the holes in the anodes (28 mm.). The solenoid of the vacuum-pump, which was run by a d.c. power supply (consumption 4 kW.), was placed on the outside of the tube. Parts of the solenoid which were in the neighbourhood of the outlets had more turns of wire than the others; in this way it was possible to obtain a sufficient intensity of the magnetic field throughout the region of the outlet openings, which was necessary for focusing the discharge without the necessity for using an internal solenoid (as in the apparatus of ref. 1). The combination of ring anodes and narrowing of the tube makes it unnecessary to have the anodes very close to the discharge tube.

The second problem was to find the dependence of the pumping speed on pressure by varying the inlet opening (that is, for different diameters of the inlet opening, for different distances between the solenoids and therefore for different intensities of the magnetic field in the region of the inlet opening). The pumping speed was deduced from the pressure drop, which was measured by a cold-cathode ionization gauge and by a McLeod gauge, both of which were connected to the evacuated region. The maximum pumping speed with an inlet opening containing three tubes of total cross-section 1·9 cm.² was 18 litres/sec. (ref. 3). In this case the distance between the solenoids was 12 mm. The maximum pumping speed for one tube of 3·2 cm.² cross-section was 34 litres/sec.; in this case the distance was 32 mm. The mean intensity of the magnetic field was 1,250 Oe., anode voltage 280 V. The dependence of pumping speed on pressure is shown in Fig. 1*a*. The pumping speed reaches a maximum for pressures of the order of 10^{-5} mm. mercury and decreases to the limiting pressure of 8×10^{-7} mm. mercury of this system.

According to present views, there are two processes involved in this pump: the transport of ions from the region of the inlet opening through the anodes into the regions of the cathodes, and sorption processes. Foster *et al.*[1] attributed the pumping effect to the transport of ions. This explanation was criticized and the effect was attributed to sorption processes, since pumping continued even after the fore-vacuum pump was disconnected. In order to show how the two effects participate in the total pumping effect the following experiments were carried out.

It was found that pumping continues (in agreement with Foster *et al.*[1]) even after the disconnexion of the rotary pump. During the measurement of the pressure drop in the evacuated system, which was carried out from the moment of closing the stop-cock connecting the discharge pump with the rotary pump, the pressure drop in the outlet openings was also measured by a gauge. This gauge showed a drop in pressure, which was, however, much smaller than

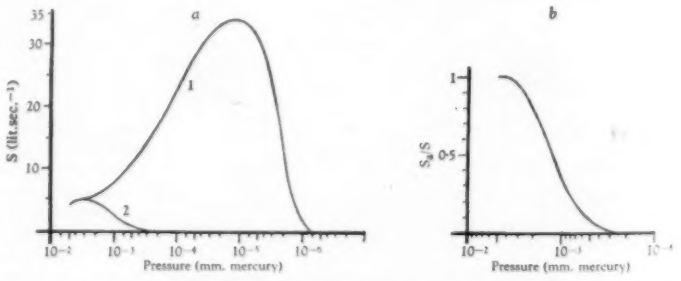

Fig. 1. Course of the pumping speed and the fraction of the total pumping effect allotted to the sorption mechanism in dependence on pressure. (*a*) Curve 1, pumping speed in the evacuated region; curve 2, pumping speed in the fore-vacuum region. (*b*) Dependence of the fraction of the total pumping effect caused by the sorption mechanism on pressure

the corresponding drop in the evacuated region. By comparing the pumping speeds in the evacuated and fore-vacuum region (Fig. 1*a*) it is found that the pumping speed S_a in the fore-vacuum region (which cannot be attributed to the transport process) as a fraction of the total pumping speed S is strongly dependent on pressure (Fig. 1*b*), and for pressures less than $2{\cdot}4 \times 10^{-4}$ mm. mercury the pump evacuates on the principle of transport and recombination of ions. It is plausible that the transport of ions becomes of importance at pressures where the mean free path becomes greater than the linear dimensions of the tube. (The values of pumping speed in the fore-vacuum region were also evaluated from the drop in pressure, that is, assuming constant volume of gas; this supposition is not quite correct, because molecules constantly flow from the high-vacuum region into the fore-vacuum region; that is why the cited values of pumping speed are only approximate.)

Finally, an experiment was carried out in which one of the anodes was inclined from its position. Hence the focused discharge was also inclined, so that a gap arose between the discharge column and the wall of the tube in the narrowed region of the tube connecting the fore-vacuum with the high-vacuum region. In the space of this gap (shaded by the inclined anode from the discharge column) molecules could enter from the fore-vacuum region into the high-vacuum region and could not be ionized on their path. By measurement it was actually found that in this case the value of the pressure in the high-vacuum region was almost equal to the pressure in the outlet openings. This effect could not be attained if the evacuation was based on sorption processes.

I wish to acknowledge the help given by Prof. V. Kunzl and Dr. L. Eckertová, of the Department of Electronics and Vacuum Physics, in the preparation of this communication for publication.

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[1] Foster, J. S., Lawrence, E. O., and Lofgren, E. J., *Rev. Sci. Instr.*, **24**, 388 (1953).
[2] Herb, R. G., Davis, R. H., Divatia, A. S., and Saxon, D., *Phys. Rev.*, **89**, 897 (1953).
[3] Páty, L., and Neužilová, R., *Czech. J. Phys.*, **8**, 746 (1958).

Optical Scanning of Liquid Concentration in Flow Studies with Porous Models

It is often convenient to employ laboratory models for the study of fluid behaviour in oil reservoirs. Typical experiments may concern the flow of two liquids miscible with one another. Basically, this calls for measurements of concentration as a function of time and position. If the liquids can be distinguished by some parameter, a corresponding method of observation may suggest itself. Thus, Beran[1] employed radioactively tagged liquids in his models, while Rifai[2], de Josselin de Jong[3] and others measured the electrical conductivity of salt solutions flowing through packed beds.

Such methods have the advantage of allowing the entire model to be monitored, but they suffer in varying degrees from lack of resolution. The porous medium is generally simulated by a packing of granular material. Retention of a realistic permeability requires that the grains be kept relatively fine; hence the main obstacle to optical scanning resides in the fact that thick packings are normally opaque.

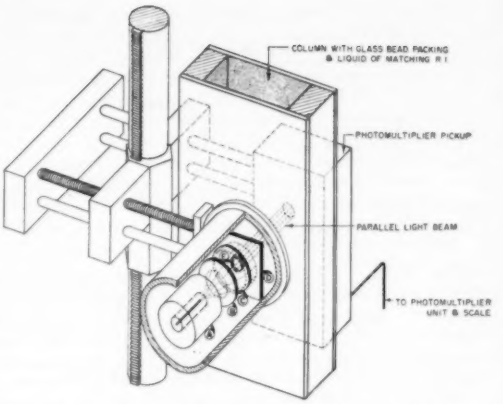
COLUMN WITH GLASS BEAD PACKING & LIQUID OF MATCHING R I
PHOTOMULTIPLIER PICKUP
PARALLEL LIGHT BEAM
TO PHOTOMULTIPLIER UNIT & SCALE

Fig. 1. Scanning unit for dispersion studies. *A*, Point source; *B*, heat filter; *C*, lens; *D*, interference filter; *E*, exit stop

In the method here described, the refractive indices of liquid and packing substance are chosen to be identical at the laboratory temperature, thus enabling a three-dimensional model to transmit light. Optically matched systems of this kind are called Christiansen filters[4,5], and are essentially a suspension of low-dispersion particles, for example, optical glass, in a highly dispersive liquid. Light transmission will be restricted to a narrow range of wave-lengths at the intersection of the two dispersion curves. The system thus acts as a monochromator the region of transmissivity of which will shift if the temperature of the liquid is changed.

In the present arrangement (Fig. 1) the above principle applies with changed emphasis. Thus, the light is rendered monochromatic prior to entry into the flooded model, which is maintained at constant temperature. At the exit face, the pencil passes through a narrow aperture into the photomultiplier unit, which registers constant intensity in the presence of a single liquid phase. When two intermiscible liquids are under study, one of the components is identified by a dye, thus allowing concentration profiles in the mixing zone to be mapped colorimetrically.

A number of applications of the Christiansen system to model studies have been suggested (Steber and Marsden[6], van Meurs and van der Poel[7], Heller[8]). The last-named employed a matrix of ground crystalline calcium fluoride, which offers the advantages of a low refractive index ($n_D = 1{\cdot}4338$) and optical isotropy. Spherical glass beads have been chosen here as an alternative packing material, being hard, chemically inert and available in bulk, besides offering surfaces which may be readily conditioned from water-wet to oil-wet preference. Anisotropy, however, now arises due to small deviations between spheres, of the order of 0·001 unit from the mean refractive index. In the present model (Table 1), where the search beam axis may cut as many as 300 glass-liquid interfaces, the above variation will suffice to cause scattering and absorption of most of the incident light. This disadvantage has been rendered unimportant by prefiltering, careful collimation and use of a sensitive detector.

The container was assembled from plates of '*CR*-39' ('Homalite') thermosetting plastic. Set up vertically, the column is first flooded with clear

Table 1. Properties of Materials used in the Model

Glass Bead Packing :
Diameter of spheres, 0·18 ± 0·03 mm.
Density, 2·86 gm./cm.³
Liquid Mixture (all data quoted for 25° C.)
Components—(*a*) chlorobenzene
(*b*) 1,2,4-trichlorobenzene
Dye, blue alkyl aminoanthroquinone
Volume proportions (*a* : *b*), 60·9 : 39·1
Density of mixture, 1·236 gm./cm.³
Viscosity of mixture, 0·81 ± 0·01 cp.
Refractive index of mixture, $n_D^{25} = 1{\cdot}5403$
Temperature gradient of R.I., $\Delta n/\Delta T = -0{\cdot}00050$/° C.
Column :
Height, 100–120 cm.
Internal cross-section, 5·0 cm. × 2·5 cm.

liquid (Table 1). In the simpler initial experiments this is displaced with an identical mixture, which in addition carries oil-soluble dye. Both phases are injected through a two-chamber unit at the base, designed to produce at time zero of the experiment a sharp and level interface between the two liquids.

The light from an argon-sealed arc is rendered yellow by a Bausch and Lomb transmission-type interference filter, with peak at 5890 Å. and half-width 80 Å. The collimated pencil is about 1·8 mm. in diameter, that is, one order of magnitude larger than the glass beads. A much narrower beam would probably register local inhomogeneities in the matrix. Lamp and photomultiplier pick-up move as a unit for scanning along two co-ordinates (Fig. 1), thus allowing transverse as well as longitudinal dispersion to be monitored in models of suitable width.

Finally, the necessary temperature control may be deduced from a curve of relative beam intensity I/I_0 versus temperature T of the clear liquid, with maximum brightness at the matching value (25° C). It is more convenient first to plot the variation of I/I_0 as a function of refractive index, with T constant. This has been done by mismatching clear mixtures in the porous model, and using the temperature gradient, $\Delta n/\Delta T$ (Table 1), to convert the result into the required curve (Fig. 2). In our arrangement, an increase in dye concentration (c) from 50 to 51 per cent will reduce I/I_0 by 1·7 per cent, and this in turn corresponds to a temperature fluctuation of less than 1° C. In a typical mixing zone of 10 cm. length (c = 10–90 per cent), the colour contrast involved should be just observable. Consequently, temperature errors of ±1° C. or more affect the measurements and must be avoided through suitable controls.

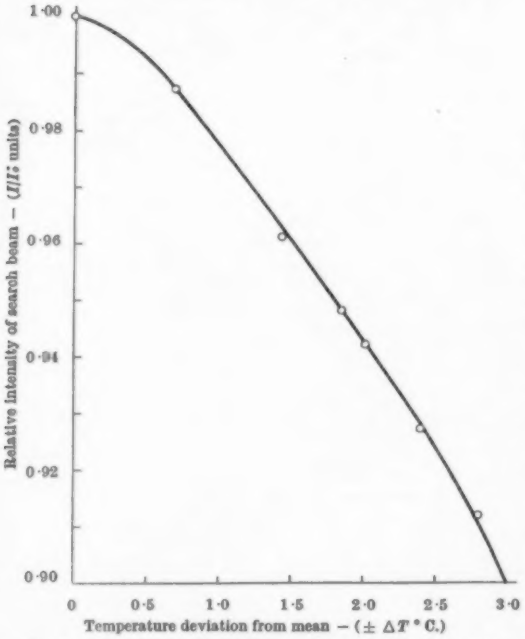

Fig. 2. Reduction in the brightness of the search beam due to temperature deviations from the matching value (for $n_D^{25} = 1{\cdot}5403$)

The method is adaptable to a variety of interesting problems in reservoir physics ; but it will be applied initially to a theoretical study of miscible flow. Solutions to the differential equations of two alternative statistical models are available[9], and the experimental method described should be well suited to furnish the data on which to base a decision.

I am indebted for much valuable advice to Mr. J. W. Young, manager of production research, Imperial Oil, Ltd., Calgary, and Prof. H. E. Gunning of the Chemistry Department, University of Alberta, Edmonton. My thanks are also due to Dr. W. N. McKay, of this Laboratory, for the viscosity determination quoted in Table 1.

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[1] Beran, M. J., Ph.D. Dissertation, Harvard University (1955).
[2] Rifai, M. N. E., Ph.D. Dissertation, University of California (Berkeley, 1956).
[3] de Josselin de Jong, G., *Trans. Amer. Geophys. Un.*, **39**, 1160 (1958).
[4] Christiansen, C., *Ann. Physik und Chemie*, **23**, 298 (1884).
[5] Summer, W., "Photosensitors", 300 (Chapman and Hall, London, 1957).
[6] Steber, J. D., and Marsden, S. S., *Producers Monthly*, **20**, 48 (1956).
[7] van Meurs, P., and van der Poel, C., *J. Petrol. Technol.*, **213**, 103 (1958).
[8] Heller, J. P., *Rev. Sci. Instr.*, **30**, 1056 (1959).
[9] Scheidegger, A. E., *Trans. Amer. Geophys. Un.*, **39**, 929 (1958).

Gallium Arsenide Tunnel Diodes

Esaki, or tunnel, diodes[1–3] in gallium arsenide have been made in this Laboratory. Zinc-doped gallium arsenide was prepared by diffusion to give a concentration of 9×10^{19} impurity atoms/cm.³ and tin pellets were alloyed on to this material to form a junction. The diameter of the pellets varied from 0·003 to 0·015 in.

Diodes made in this manner have shown negative resistances of between 0·1 Ω and 4 Ω, with a wide range of peak currents from 70 m.amp. to 7 amp. The highest ratio of peak to valley currents observed was 30 : 1, and the average value was about 15 : 1. The voltages at which the peak and valley currents occurred were not constant but varied from device to device, from 0·13 to 0·50 V. for the peak current, and from 0·35 to 0·55 V. for the valley current. These variations can be partially explained by the presence of series resistance of about 0·1 Ω, probably in the contacts to the devices.

The diodes were found to switch across the unstable negative resistance region in a time of less than 1 mμsec., this being the limit of the measuring equipment. When the diodes were mounted in a strip-line designed to resonate at a frequency of about 400 Mc./s., relaxation oscillations were observed. The fundamental frequency varied with d.c. bias voltage over the range 300–560 Mc./s. and in all cases harmonics were seen up to 1 kMc./s., which

was the upper limit of the receiver. 5 milliwatts output power was obtained.

We wish to thank the Admiralty for permission to publish this communication.

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[1] Esaki, L., *Phys. Rev.*, **109**, 603 (1958).
[2] Sommers, H. S., *Proc. Inst. Rad. Eng.*, **47**, 1201 (1959).
[3] Gremmelmaier, R., and Henkel, H., *Z. Naturforsch.*, **14**, *a*, 1072 (1959)

GEOPHYSICS

Micropulsations in the Earth's Magnetic Field Simultaneous with Pulsating Aurora

Some preliminary results have been obtained from an investigation being undertaken at College, Alaska, concerning the correspondence of the Earth's magnetic field micropulsations[1] with pulsating aurora for the purpose of explaining the physical mechanism giving rise to the phenomena. Fig. 1 illustrates the nightly coincidence of micropulsations, measured with a 6-ft. diameter magnetic north axis loop antenna of 21,586 turns and 3914-Å. auroral pulsations from 70° of sky measured with a lens, interference filter, photo-multiplier system. Fig. 2 shows an occasion of simultaneous oscillations from the two phenomena.

Messrs. A. Belon, C. Deehr, M. Rees and G. Romick, of the Geophysical Institute Optics Division, have aided me in the development of the experiments.

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[1] Campbell, W. H., *J. Geophys. Res.*, **64**, 1819 (1959).

An Expanding Earth with Loss of Gravitational Potential Energy

Recently, and independently, Carey[1] and Heezen[2] have concluded that the Earth is expanding. The

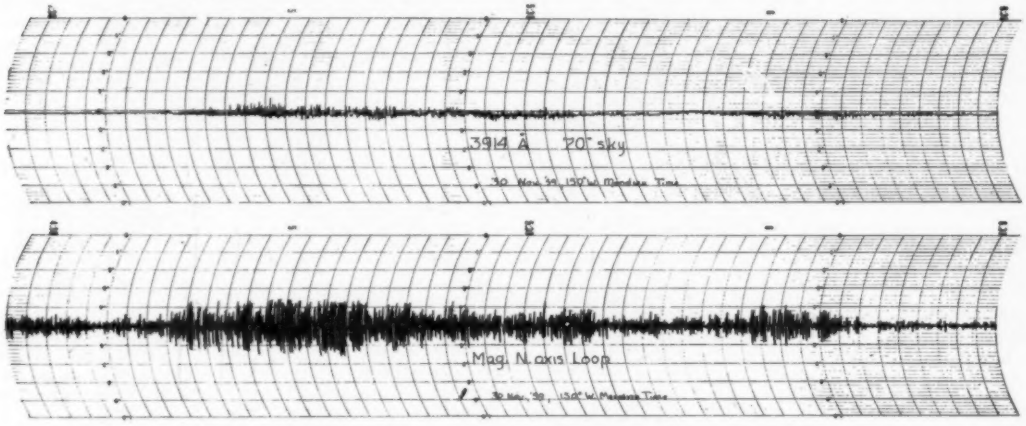

Fig. 1. Coincident pulsating aurora and magnetic micropulsations (12γ full scale)

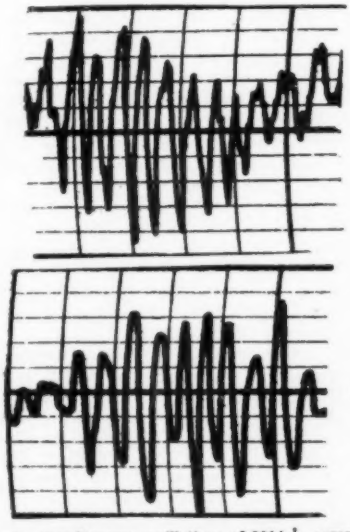

Fig. 2. Simultaneous oscillations of 3914 Å. aurora and micropulsations in the magnetic north–south direction

first question asked of such a hypothesis usually takes the form of an inquiry as to the source of energy for such an expansion. This question is based on the assumption that the gravitational potential energy of the Earth is inversely proportional to its radius, and the assumption in turn is presumably based on the well-known equation (see, for example, Loney's "Statics", p. 359).

$$E = -\frac{3}{5}\frac{GM^2}{a}$$

where E is the gravitational potential energy, G the gravitational constant, M the mass and a the radius of the Earth. However, the equation refers only to a sphere of uniform density, and one possibility that appears to have been overlooked is that a non-uniform Earth can expand and lose gravitational potential energy at the same time.

The following expressions for density ρ_r versus radius r in the Earth have been obtained by fitting linear density distributions of the form: $\rho_r = \rho_0 (1 - kr)$ to each section of Bullen's[3] model B of the Earth:

$0 < r < 1{\cdot}250 \times 10^8$ cm.	$\rho_r = 17{\cdot}900(1-1{\cdot}29162 \times 10^{-8}r)$
$1{\cdot}250 < r < 1{\cdot}389$,,	$\rho_r = 42{\cdot}078(1-5{\cdot}14625 \times 10^{-8}r)$
$1{\cdot}389 < r < 3{\cdot}471$,,	$\rho_r = 13{\cdot}403\,(1-7{\cdot}53631 \times 10^{-10}r)$
$3{\cdot}471 < r < 6{\cdot}291$,,	$\rho_r = 7{\cdot}505(1-7{\cdot}69678 \times 10^{-10}r)$
$6{\cdot}291 < r < 6{\cdot}338$,,	$\rho_r =$ constant $= 3{\cdot}34$
$6{\cdot}338 < r < 6{\cdot}371$,,	$\rho_r =$ constant $= 2{\cdot}79$

If spherical symmetry is assumed these distributions give the right mass, $5{\cdot}977 \times 10^{27}$ gm., and a slightly high moment of inertia, $8{\cdot}120 \times 10^{44}$ gm. cm.^{2}; the discrepancy between this value and the generally accepted one is immaterial for the purposes of the present argument. The gravitational potential energy of an Earth with the above density distributions is $-2{\cdot}47949 \times 10^{39}$ ergs.

If in the past the density distribution in the Earth was of the form $\rho_r = \rho_0(1 - kr^2)$ from $0 < r < a$, where a is its past radius, then assuming that the mass has remained constant and that the surface density of the Earth was 2·85 gm. c.c.$^{-1}$, one model with a higher potential energy but a smaller radius than the present Earth is $a = 6{\cdot}27 \times 10^8$ cm. and $\rho_r = 10{\cdot}197(1 - 1{\cdot}83275 \times 10^{-18}\, r^2)$. The potential energy of this model is $-2{\cdot}47464 \times 10^{39}$ ergs and its moment of inertia is $8{\cdot}036 \times 10^{44}$ gm. cm.^{2}. If there are no external or internal sources of energy the Earth will tend to proceed from a higher state of potential energy to a lower state, and one way it could do this would be to expand to its present form. Thus expansions of the order of 100 km. seem quite possible without postulating any source of energy. The Earth will, in fact, lose energy during the expansion.

If it is assumed that the mass of the Earth has remained constant and that $\rho_a = 2{\cdot}85$ gm. c.c.$^{-1}$ then Earth models of smaller radii but higher potential energies can be found for any value of $n \geqslant 1$, where $\rho_r = \rho_0(1 - kr^n)$. The higher the value assumed for n the greater is the possible expansion. Furthermore, for each value of n the range of models with likely density distributions covers models with higher or lower moments of inertia than the present Earth. If density distributions which, on present evidence, seem improbable are postulated, then expansions of the order of 1,000 km. are possible. Expansions are also possible even if the region $0 < r < 3{\cdot}471 \times 10^8$ cm. is kept constant and only the mantle and crust are changed.

In this communication no attempt has been made to postulate possible mechanisms. These, and other possible models, will be discussed in a fuller account elsewhere.

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[1] Carey, S. W., "The Tectonic Approach to Continental Drift", Continental Drift Symposium, 177 (University of Tasmania, Hobart, 1958).

[2] Heezen, B. C., "Paleomagnetism, Continental Displacements, and the Origin of Submarine Topography", International Oceanographic Congress Preprints, 26 (Amer. Assoc. Adv. Sci., Washington, 1959).

[3] Bullen, K. E., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **6**, 50 (1950).

BIOPHYSICS

Effect of Ultra-violet Light on the Shape and Size of Deoxyribonucleic Acid

Several investigators[1,2] have found that the viscosity of solutions of deoxyribonucleic acid is reduced by irradiation with ultra-violet light of 254 mμ. This change could be the result either of main-chain scission which reduces the length of the molecules or of an increase in flexibility which allows the molecule to take up a more coiled configuration and thereby reduces the volume occupied in solution, as measured by the radius of gyration.

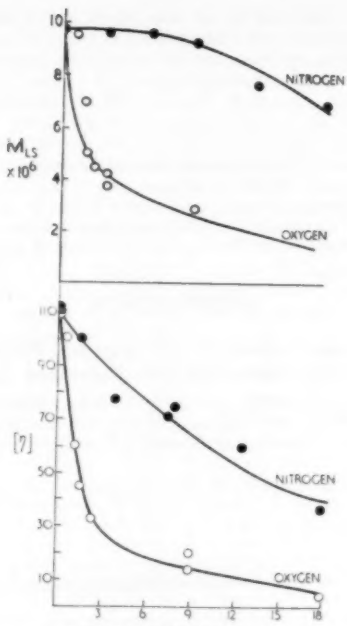

Fig. 1. Changes in the weight average molecular weight (M_{LS}) and in the intrinsic viscosity ([η] in dl./gm.) of deoxyribonucleic acid following irradiation with ultra-violet light of 254 mμ at the rate of 1×10^3 ergs/cm.^{2}/sec. The polymer was at a concentration of 0·01 gm./dl. and either oxygen or purified nitrogen was bubbled through the solution before and during the irradiation

We have carried out simultaneous measurements of the molecular weight by light scattering and of the viscosity in a Couette viscometer on solutions of deoxyribonucleic acid irradiated with a 44-watt 'Hanovia 769/67' mercury resonance lamp in a 'Vycor' envelope. The water-cooled irradiation vessel was specially constructed to allow total immersion of the lamp in the deoxyribonucleic acid solution and gas bubbling through the solution during the irradiation. The deoxyribonucleic acid was obtained from viable salmon sperm and contained less than 0·1 per cent of protein. Different preparations, varying in molecular weight, were used for the experiments shown in Fig. 1 and in Table 1. All measurements were made in 0·2 *M* saline containing citrate buffer.

Fig. 1 shows that, in the absence of oxygen, the first effect of ultra-violet light of 254-mμ wavelength is to reduce the viscosity without altering the molecular weight. As the dose is increased the molecular weight falls, showing that the molecule is severed, but the principal effect remains one of coiling.

If oxygen is present, then the molecular weight falls immediately; but the change in viscosity is very much greater than that due to the reduction in molecular weight alone. The introduction of oxygen gives rise to a second process, main-chain scission, without decreasing the amount of coiling that is produced when the irradiation is done under pure nitrogen.

This oxygen effect is not due to attack by ozone since the 'Vycor' filter effectively excludes the oxygen-

Table 1. Effect of Added Substances on Ultra-violet-induced Changes of Molecular Weight (M_{LS}) and Radius of Gyration (ρ_Z in Å.) of Deoxyribonucleic Acid

Dose (ergs/cm.^{2} × 10^3)	No additive		Dimethylaniline 10^{-3} M		Glutathione 10^{-4} M		Glucose 10^{-1} M		Catalase 0·01 per cent	
	M_{LS}	ρ_Z	M_{LS}	ρ_Z	M_{LS}	ρ_Z	M_{LS}	ρ_Z	M_{LS}	ρ_Z
None	6·7	2,420	7·7	2,600	6·3	2,400	6·3	2,420	7·7	2,400
14 (Nitrogen)	6·0	1,240	5·7	1,370	6·1	1,570	5·5	1,450	7·7	1,750
3·6 (Oxygen)	2·5	1,060	5·7	1,980	5·1	1,610	7·3	2,120	4·5	1,850

absorbing bands of ultra-violet light. Experiments in which oxygen that had been passed over the 'Vycor' lamp and was then bubbled through a solution of deoxyribonucleic acid failed to reveal any degradation.

Coiling of deoxyribonucleic acid is produced when the specific hydrogen bonding between the bases in the twin helix is disrupted[3]. Our results would suggest that this structure is disorganized by ultra-violet light before there is any extensive photochemical degradation of the bases. Internal conversion can lead to the degradation of excitation energy to thermal energy, and it would appear that the increase in the oscillational degrees of freedom brings about this change of shape. Photolysis of the pyrimidine or purine bases would not decrease the molecular size except by initiating further reactions such as hydrolysis.

Direct interaction between an oxygen molecule and the singlet excited state to form peroxides which are further decomposed by the ultra-violet light can best explain the main-chain scission in the presence of oxygen. Photochemically produced OH radicals have been shown[4] to reduce the viscosity of solutions of deoxyribonucleic acid and we were able to show by light-scattering measurements that this effect was due mainly to main-chain scission.

Dimethylaniline, glutathione and glucose were all found to protect the molecular weight and radius of gyration of the irradiated deoxyribonucleic acid against the oxygen effect (see Table 1), presumably by competitively reacting with the degradative free radicals produced via the peroxide. Protection by catalase was observed and is probably due to the decomposition of the peroxide in such a way that it does not give rise to degradative free radicals. Poisoning of the catalase by potassium cyanide caused a loss in protective activity. In the absence of oxygen none of these substances reduced either the coiling or the main-chain scission process significantly, except, oddly, catalase.

A puzzling feature is that the biological effects produced by ultra-violet light have not been found to be influenced by oxygen. Possibly cell constituents act as protective agents, and from this it would follow that cellular lesions in so far as they are due to attack on deoxyribonucleic acid follow from an interference with the hydrogen bonding between the bases and not from a degradative reaction. Doses needed to produce detectable coiling are very large when compared with those necessary to produce changes in cells. Probably the number of hydrogen bonds that have to be broken to produce sufficient coiling to be measured by physicochemical techniques is very much greater than that necessary for interfering with the biological function of deoxyribonucleic acid.

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[1] Hollaender, A., and Oliphant, J., *J. Bact.*, **48**, 447 (1944).
[2] Errera, M., *Biochim. Biophys. Acta*, **8**, 30 (1952).
[3] Thomas, C. A., and Doty, P., *J. Amer. Chem. Soc.*, **78**, 1854 (1956).
[4] Butler, J. A. V., and Conway, B. E., *Proc. Roy. Soc.*, B, **141**, 562 (1953).

Blood Flow through Narrow Tubes

In the course of an extensive investigation (with Prof. A. C. Burton at the University of Western Ontario) of the hæmodynamics of perfused vascular beds, it was found necessary to examine the somewhat simpler case of blood flow in narrow glass tubes. Accordingly, the pressure-flow curves in tubes of radii 50–750μ for human erythrocytes suspended in standard acid–citrate–dextrose were measured at 25·5° C. over a wide range of hæmatocrits[1–4]. A typical set of such pressure-flow curves is shown in Fig. 1. The curves were found to approach linearity at moderate flow rates; if the linear segments are extrapolated back, they intersect the positive pressure axis at finite values and then converge to a common 'nodal point' on the negative flow axis. However, in the non-linear region at very low flow rates, the curves appear to converge toward the origin. There is no evidence of any yield stress or intercept on the positive pressure axis. All the curves can be represented by an equation of the form:

$$\dot{Q} = MP - B[1 - \exp(-kP)] \qquad (1)$$

where $\dot{Q}$ is the volume rate of flow and P the pressure gradient; the parameters M and k depend on both tube radius and hæmatocrit, whereas the nodal point B depends only on the tube radius[3]. Pressure-flow curves of similar shape have been obtained by other workers and can be inferred from the apparent viscosity data of Kümin[5].

The results obtained from the basic pressure-flow measurements were used to plot the rate of shear at the wall versus the shear stress (consistency curves). It can be shown[3] that the shear-rate is given by:

$$F = \frac{1}{\pi R^3}\left[3\dot{Q} + P\frac{d\dot{Q}}{dP}\right] \qquad (2)$$

or: $$F = \frac{4\dot{Q}}{\pi R^3} + \frac{B}{\pi R^3}\left[1 - (1 + kP)\exp(-kP)\right] \qquad (3)$$

and can be plotted from (3) as a function of the shear stress $PR/2$. The first term in (3) is the shear-rate

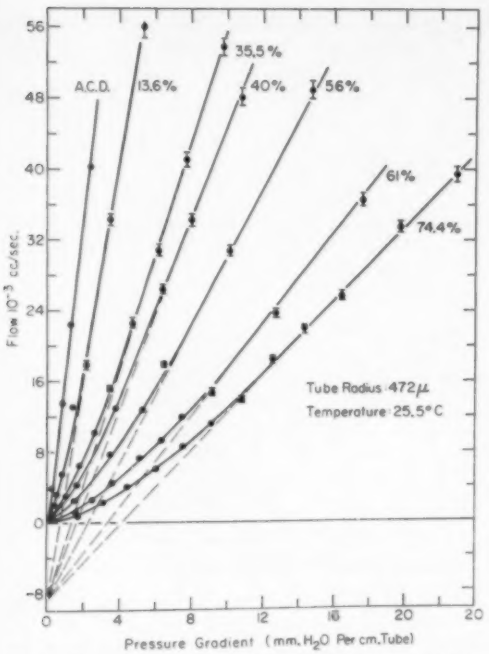

Fig. 1. Pressure-flow curves for human red cells suspended in acid-citrate-dextrose for various hæmatocrits as indicated in volume per cent

for a Newtonian liquid while the second term arises from the non-linearity of the pressure-flow curves. A set of such consistency curves for three hæmatocrits is shown in Fig. 2. They have approximately the same shape as the pressure-flow curves, and become linear at shear stresses greater than about 20 dynes/cm. From the data of Kümin[5], it is possible to plot what might be called the 'apparent shear rate' as a function of shear stress as shown in Fig. 3. This is a plot of the usual consistency variables (for a fluid that obeys Poiseuille's law), so that the contribution of the second term in equation 3 is not present. The error introduced by this approximation is less than 10 per cent, and it does not affect the basic curve shape. The important features of all these curves are the non-linearity below about 20 dynes/cm., and the absence of any finite intercept on the stress axis.

Equation 1 can be given a phenomenological interpretation by assuming that the fractional change in differential fluidity is proportional to the change in shear stress[4]. Such a fluidity change can be interpreted physically as a structural re-organization of the fluid with increasing flow-rates that arises from the axial accumulation of the red cells in a variable shear field[3].

The Fahraeus–Lindqvist effect appeared very prominently in the data of Kümin[5] and also in our own experiments[3,4]. The tube radius at which the apparent viscosity is reduced 10 per cent below the asymptotic value for large tubes was found to vary from 130μ at 10 per cent hæmatocrit to 633μ at 80 per cent. If the effect is interpreted as a sigma phenomenon[4], the average thickness of the unsheared laminæ was found to vary from 3·5μ at 10 per cent hæmatocrit to 34μ at 80 per cent. If, on the other hand, the effect arises from the presence of a cell-free marginal zone[7,8], it was found that its thickness must be 6μ at 10 per cent hæmatocrit, 3μ at 40 per cent, and only 1·5μ at 80 per cent. Such a cell-free marginal zone could also result from the axial accumulation of the red cells. It is difficult to determine the relative importance of the marginal zone and the

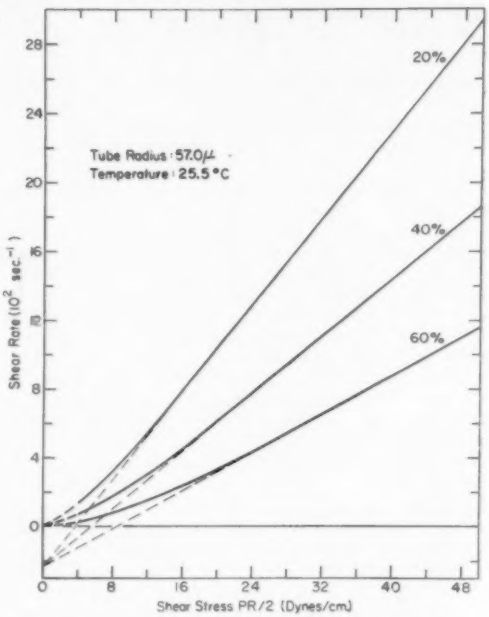

Fig. 2. Consistency curves for human red cell suspensions of three hæmatocrits. The broken curves near the origin represent the only extrapolation beyond the measurements

Fig. 3. Consistency curves for defibrinated ox blood calculated from data of Kümin

sigma phenomenon in producing the Fahraeus–Lindqvist effect.

On the basis of some measurements of A. L. Copley, Scott Blair[9] and Reiner and Scott Blair[10] have recently suggested that the rheological properties of blood can be described in terms of a theory put forward by Casson[11] for varnish and ink. This theory is applicable to substances the consistency curves of which intersect the stress axis at a yield value. However, the consistency curves which we have obtained for blood do not seem to intersect the stress axis at a finite value, but rather become non-linear below 20 dynes/cm. and point toward the origin. The lowest shear stress that was reached in Copley's measurements was 100 dynes/cm., and so the non-linearity of the consistency curves was not detected. Thus it does not appear that Casson's theory would provide a satisfactory description of the flow properties of blood at very low shear stresses if no yield value exists.

I wish to thank Dr. M. G. Taylor, now at the University of Sydney, for several helpful discussions, and Mr. J. E. Baptist, of this laboratory, for his help with the calculations.

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[1] Haynes, R. H., *Canad. Biol.*, **15**, 255 (1956).
[2] Haynes, R. H., and Burton, A. C., "Proceedings of the First National Biophysics Conference", ed. by Quastler, H., and Morowitz, H. J., 452 (Yale University Press, 1959).
[3] Haynes, R. H., and Burton, A. C., *Amer. J. Physiol.*, **197** (5), 943 (1959).
[4] Haynes, R. H., "The Rheology of Blood", doctoral dissertation (University of Western Ontario, London, Canada, 1957).
[5] Kümin, K., Inaugural Dissertation, Universität Bern (Paulus-druckerei, Freiburg in der Schweiz, 1949).
[6] Dix, F. J., and Scott Blair, G. W., *J. App. Phys.*, **11**, 574 (1950).
[7] Taylor, M. G., *Phys. Biol. Med.*, **3**, 273 (1959).
[8] Taylor, M. G., *Aust. J. Exp. Biol. Med. Sci.*, **33**, 1 (1955).
[9] Scott Blair, G. W., *Nature*, **183**, 613 (1959).
[10] Reiner, M., and Scott Blair, G. W., *Nature*, **184**, 354 (1959).
[11] Casson, N., "The Rheology of Disperse Systems", ed. by Mill, C. C., Chap. 5 (Pergamon Press, London, 1959).

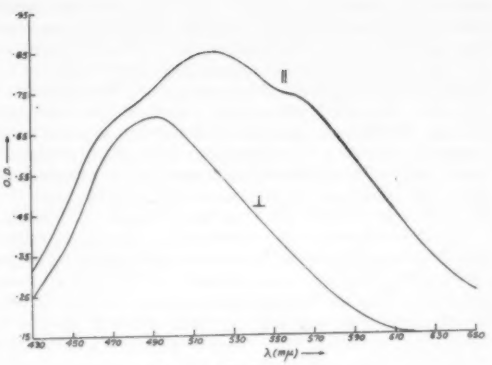

Fig. 1

transition $d_{z^2} \rightarrow d_{xy}$, then the result may mean that the z-axis of the molecular field coincides with the long axis of the crystal.

The crystal spectrum of the chloride and also of the fluoride was found to be identical with that of the bromide, indicating that the anion was outside the co-ordinating sphere and had no perturbing action on the copper d-orbitals, which is also corroborated by the chemical properties[2]. In solution, all these compounds have one broad absorption band and show no structure resembling a crystal spectrum. Further, the structure could be detected with extremely thin crystals only. As the thickness of the crystal was increased, the structure of the band became increasingly less well defined.

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[1] Chakravorty and Basu, *Nature*, **184**, 50 (1959).
[2] Rây and Bagchi, *J. Ind. Chem. Soc.*, **16**, 617 (1939).

CHEMISTRY

Crystal Spectrum of Copper Biguanides

IN a previous communication[1] it was shown that the absorption spectrum of a microscopic crystal of copper-ethylene-diamine-*bis*(acetylacetone) consists of three bands as predicted by the ligand field theory. Copper biguanides form transparent reddish crystals[2], very convenient for measurement in our experimental arrangement. In Fig. 1 are given the spectrophotometric optical density (*O.D.*) vs. wave-length (λ) curves for a thin crystal of copper *bis*-biguanide dibromide dihydrate with light polarized along the long axis of the crystal and at right angles to it, respectively. It is evident that the first curve consists of three bands, but the sharpness and fine structure are not as well defined as was the case with copper-ethylene-diamine-*bis*(acetylacetone). The decomposition of the curve into its component bands shows three peaks at 485 mμ, 540–550 mμ and 600 mμ. When the light was polarized at right angles to the long axis of the crystal, the absorption spectrum was found to consist of two bands, with peaks at 485 mμ and 550 mμ, the peak at 600 mμ being missing. If we associate the band of longest wave-length with the

Structure of Lithium Methoxide

RECENTLY, Bradley has proposed a theory which correlates and predicts the structures of metallic alkoxides[1]. At the moment, Bradley's theory cannot be fully developed because of the lack of direct structural information. In particular, it is not known whether lithium methoxide is ionic, as generally assumed, or whether it is a giant polymer[2]. This communication describes the structure of lithium methoxide, which is shown to be similar to that of lithium hydroxide[3]. Although the structure has some features reminiscent of a giant two-dimensional polymer, it would normally be considered ionic.

Lithium methoxide was prepared by solution of the metal in methanol. Excess methanol was pumped off, and specimens of the resultant powder transferred to thin-walled 'Pyrex' capillaries which were sealed with 'Picien' wax. All operations were carried out in a dry box. Powder photographs were taken with a 3-cm. 'Unicam' oscillation camera. The photographs showed that the unit cell was tetragonal with $a = 3{\cdot}55_2$ and $c = 7{\cdot}68_7$ Å. Systematic absences corresponded to the space group $P4/nmm$ (No. 129). The density calculated on the assumption of two formula units in the cell was 1·30 gm./c.c. The

observed density, which could not be accurately determined because the powder was so hygroscopic, lay between 1·2 and 1·5 gm./c.c. The lithium ions lie at $\pm$ ($\frac{1}{4}$, $\frac{3}{4}$, 0), the oxygen atoms at $\pm$ ($\frac{1}{4}$, $\frac{1}{4}$, u_1), and the carbon atoms at $\pm$ ($\frac{1}{4}$, $\frac{1}{4}$, u_2). The methoxide ion has four-fold symmetry so that the hydrogen atoms, which were ignored in the analysis, presumably adopt a statistical arrangement to conform with this symmetry. The parameters u_1 and u_2 were determined by trial and error and found to be $0{\cdot}10_5$ and $0{\cdot}29$ respectively. Thus the O—CH_3 distance is 1·42 Å.

If the structure is viewed in a direction normal to the four-fold axis, it is seen to consist of layers *ABCBAABCBA* . . . , with $A = CH_3$, $B = O$ and $C = Li$. The methyl groups are separated by 4·09 Å. in the [*c*] direction and by only 3·55 Å. in the [*a*] and [*b*] directions. The oxygen atoms are bonded to a methyl group on one side and are co-ordinated to four lithium ions on the other to form square pyramids with oxygen atoms at the centre. The Li . . O distance is 1·95 Å. and the LiOLi angle 80·3°. The lithium ions are surrounded by a tetrahedron, flattened in the [*c*] direction, of oxygen atoms. The OLiO angles are 131·1° and 101·7°. The Li . . Li distance is 2·51 Å. and the nearest O . . O contact 2·99 Å. The LiOCH_3 angle is 114·5°.

A full account of this work and of further physico-chemical measurements on this and other alkali metal methoxides will be published elsewhere.

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[1] Bradley, D. C., *Nature*, **182**, 1211 (1958).
[2] Bradley, D. C., and Faktor, M. M., *Nature*, **184**, 55 (1959).
[3] Ernst, Th., *Z. phys. Chem.*, B, **20**, 65 (1933). Dachs, H., *Z. Krist.*, **112**, 60 (1959).

New Univalent Cobalt Complexes of the $[Co^I(CN)_5X]$ Type

Hume *et al.*[1] found that the aquopentacyanocobaltate(III) and cobaltate(II) complexes were irreversibly reduced in one step to the cobalt(I) state polarographically in the cyanide medium, and the cobalt(I) complex was neither reducible nor oxidizable at the dropping mercury electrode.

Recently, the similar cobaltate(III) complexes of pentacyano type, $K_{2+n}[Co^{III}(CN)_5X]$, were also found to be reduced irreversibly in one or two steps to the univalent cobalt complexes at the dropping mercury electrode[2], where X denotes the ligand, Cl^-, Br^-, I^-, SCN^-, N_3^-, $S_2O_3^{2-}$, NO_2^-, or SO_3^{2-}.

In order to clarify the structure and the co-ordination number of this univalent cobalt complex, a number of attempts have been made to isolate the complex from the solution, but have failed mainly due to the great solubility of the complex in alcohol and water.

From the following two reasons, however, it was concluded that the univalent cobalt complex has a similar structure to the original oxidized form, that is, the hexa-co-ordinated formula, $[Co^I(CN)_5X]$.

(1) It was ascertained in advance polarographically that the yellow solution of the univalent cobalt complex was easily obtained from the reduction of the pentacyanocobaltate(III) ion by 3 per cent sodium amalgam in an atmosphere of nitrogen. No deposition of metallic cobalt was found in any case. From this solution of the cobaltate(I) complex, the pentacyanocobaltate(II) complex, $K_3[Co^{II}(CN)_5]\cdot H_2O$ (ref. 3), was always isolated in an atmosphere of nitrogen as bright violet crystals by addition of ethanol in portions with respect to all the pentacyanocobaltate(III) complexes. This fact means that the pentacyano structure was not destroyed and was still maintained in the univalent cobalt complex.

(2) Generally, all these cobaltate(I) complexes were oxidized by air to the cobalt(III) state on exposure. By pursuing the increase of the wave-height with time, however, it was found that there existed considerable differences among the rates of oxidization, depending upon the kind of ligand, X. For example, the cobaltate(I) complex obtained from the reduction of azido- or nitro-pentacyanocobaltate(III) ion was seen to be instantly oxidized to the cobalt(III) state, on being exposed to the air. On the other hand, the oxidation-rate of the cobaltate(I) complex obtained from the other pentacyanocobaltate(III) ions was found to be small, though the cobaltate(I) complex was still oxidized slowly in open air. This fact implies that the sixth ligand, X, is still co-ordinated about the central cobalt(I) atom in the univalent cobalt complex.

The re-oxidized product obtained from these univalent cobalt complexes was also identified as a hydroxypentacyanocobaltate(III) complex, $K_3[Co^{III}(CN)_5OH]$ (yellow crystals). This is due to the alkalinity which resulted from the sodium amalgam used. Therefore, the bond between Co^I and the sixth ligand, X, is considered not to be so strong. Hence, in a solid state, it might be possible that the penta-co-ordinated complex, $K_4[Co^I(CN)_5]$, can exist rather than the hexa-co-ordinated complex, $K_4[Co^I(CN)_5OH_2]$, being considered analogous to the case of the penta-*iso*nitrilo complexes[4], $[Co^I(NCR)_5]Y$ (yellow crystals), where R indicates phenyl-, *p*-tolyl-, or *o*-tolyl-radical and Y, the ion, ClO_4^-, I^-, NO_3^-, or ClO_3^-.

Accordingly, the processes of the electrode reaction for the pentacyanocobaltate(III) complexes were considered to be as follows. For the halogeno complexes :

$$[Co^{III}(CN)_5X]^{3-} + e^- \longrightarrow [Co^{II}(CN)_5X]^{4-} \quad \text{1st step}$$
$$[Co^{II}(CN)_5X]^{4-} + e^- \longrightarrow [Co^{I}(CN)_5X]^{5-} \quad \text{2nd step}$$
$$[Co^{I}(CN)_5X]^{5-} + H_2O \longrightarrow [Co^{I}(CN)_5OH_2]^{4-} + X^-$$
$$X = Cl^-, Br^-, I^-$$

For the thiocyanato complex (in the pentacyano complex, $K_3[Co(CN)_5SCN]$, the ligand, SCN^-, is bound to the central cobalt(III) atom by sulphur, while in the ammine complexes, for example $[Co(NH_3)_5NCS]Cl_2$, the ligand, NCS^-, is co-ordinated by nitrogen atom. Therefore, in this case, the equation of the complex can scarcely be considered) :

$$[Co^{III}(CN)_5SCN]^{3-} + e^- \longrightarrow [Co^{II}(CN)_5SCN]^{4-} \quad \text{1st step}$$
$$[Co^{II}(CN)_5SCN]^{4-} + e^- \longrightarrow [Co^{I}(CN)_5SCN]^{5-} \quad \text{2nd step}$$

For the azido-, nitro-, thiosulphato- and sulphito-complexes :

$$[Co^{III}(CN)_5X]^{(3+n)-} + 2e^- \longrightarrow [Co^{I}(CN)_5X]^{(4+n)-} \quad \text{1st step}$$
$$X = N_3^-, NO_2^-, S_2O_3^{2-}, SO_3^{2-}$$

The resulting pentacyanocobaltate(I) complexes did not give the anodic wave at all. Namely, all the above electrode reactions are irreversible polarographically, though the reversibility of the reduction waves looks fairly large in appearance.

The mechanism of the electron transfer and the electronic configuration for the above reactions are believed to be as follows. The acceptance of the electrons occurs in two stages:

$$d^2d^2d^2[D^2SP^2] \xrightarrow{e^-} d^2d^2d^2[D^2SP^2]d^1 \longrightarrow$$

$$d^2d^2d^2d^1[DSP^2PD] \xrightarrow{e^-} d^2d^2d^2d^2[DSP^2PD]$$

First, the electron is accepted into the 4*d* peripheral orbitals temporarily, where it is held loosely, since the 3*d* atomic orbitals of Co(III) atom are fully occupied. After that the consecutive change of the electronic configuration and the placement of the electron in the stable 3*d* orbitals occur slowly. Therefore, this electrode process requires fairly high overpotential. In the second stage, the electron is accepted into the vacant 3*d* orbital directly. Thus, the difference of the electronic configuration in the oxidized and the reduced forms is considered to be one of the main causes for irreversibility of the electrode reactions.

I wish to thank Prof. R. Tsuchida for helpful discussions.

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[1] Hume, D. N., and Kolthoff, I. M., *J. Amer. Chem. Soc.*, **71**, 867 (1949).
[2] Maki, N., Fujita, J., and Tsuchida, R., *Nature*, **183**, 458 (1959).
[3] Adamson, A. W., *J. Amer. Chem. Soc.*, **73**, 5710 (1951).
[4] Malatesta, L., and Sacco, A., *Z. anorg. allg. Chem.*, **273**, 247 (1953)

Carbonates of Uranium

MILLER and co-workers[1] for the first time isolated uranyl carbonate, prepared by the action of carbon dioxide under pressure on uranium trioxide, ammonium and sodium uranates. A different process was followed by Chernyaev and co-workers[2], who prepared the compound by the treatment of aqueous and alcoholic solutions of uranyl nitrate with carbon dioxide under pressure or aqueous suspensions of uranium oxide, UO_3, under normal conditions. A simple and convenient method has been developed by us for the preparation of the compound. The hitherto unreported compound uranium (IV) oxycarbonate, the preparation of which is described below, on exposure to dry air undergoes simultaneous oxidation and dehydration, resulting in the formation of the compound UO_2CO_3, 2·5 H_2O, the progress of the oxidation being indicated by the gradual change of colour from dark green to bright yellow. This hydrated uranyl carbonate, when heated *in vacuo* at 160°, gives the anhydrous compound UO_2CO_3, which is stable up to 500° C.

Uranyl ion undergoes photoreduction when exposed to sunlight in the presence of reducing agents such as alcohol or formic acid. When ammonium carbonate is added to a photochemically reduced solution of uranyl nitrate or formate, a green precipitate is obtained in a fine state, which on washing turns dark green. Analysis of the freshly prepared moist compound was carried out after ammonia had been tested for and found to be absent. The uranium to carbonate ratio was found to be 1 : 1 by semimicro-combustion method. The water content was found to vary from time to time due to the moist compound being used for analysis. The total uranium content was estimated as U_3O_8 and also by the Jones reductor method. That all the uranium exists as uranium (IV) was proved by directly titrating the solution of the compound in 2 *N* sulphuric acid against standard potassium permanganate. On account of the extreme proneness of the compound to oxidation and hydrolysis at high temperature, and also our lack of equipment for isothermal dehydration at low temperature, it has not been possible to get the compound either in the anhydrous state or in a definite state of hydration.

The isolation of uranium (IV) oxycarbonate offers a new route for the syntheses of uranium (IV) compounds[3]. Investigations are proceeding with the view of preparing some new compounds of uranium as well as the dioxide[4].

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[1] Miller, P. D., Pray, H. A., and Munger, H. P., Rep. A.E.C.D. (1949).
[2] Chernyaev, I. I., Golovnya, V. A., and Ellert, G. V., *Zhur. Neorg. Khim.*, **1**, 35, 2726 (1956).
[3] Sahoo, B., and Patnaik, D., *Curr. Sci.* (in the press).
[4] Sahoo, B., and Patnaik, D., *Curr. Sci.* (in the press).

Photolysis of 1 : 3-Dichlorotetrafluoroacetone

THE primary act in the photolysis of acetone at room temperature has been shown by a number of workers to be the production of an acetyl and a methyl radical[1]. It is believed that the photolysis of hexafluoroacetone is a simpler process in which only trifluoromethyl radicals and carbon monoxide are formed[2]. Hexafluoroacetone is thus an unambiguous photochemical source of trifluoromethyl radicals, and the close structural similarity of 1 : 3-dichlorotetrafluoroacetone has prompted an investigation into its suitability as a source of difluorochloromethyl radicals.

The near ultra-violet absorption spectrum of 1 : 3-dichlorotetrafluoroacetone shows a broader and more intense band than does that of acetone ($\lambda_{max.}$ 2,900–3,100, $\varepsilon_{max.}$ 80, cf. acetone $\lambda_{max.}$ 2,700–2,800, $\varepsilon_{max.}$ = 16). A preliminary study of the photolysis of this ketone, at room temperature, using a high-pressure mercury lamp, has indicated that the primary step in this case is also dissociation into two identical radicals and a molecule of carbon monoxide. This conclusion is based upon the absence among the secondary products of reaction of any species which could have arisen from the difluorochloroacetyl radical, for example, tetrafluorodichlorodiacetyl.

The photodecomposition of dichlorotetrafluoroacetone is, however, more complex than that of hexafluoroacetone because the chlorodifluoromethyl radicals formed in the primary step are capable of abstracting chlorine atoms present in the undissociated ketone. Thus in the products of photolysis there appears, in addition to the *sym.*-dichlorotetrafluoroethane formed by the dimerization of chlorodifluoromethyl radicals, a smaller concentration of dichlorodifluoromethane. These two compounds have been isolated from the reaction products by means of gas chromatography, and their identity has been confirmed by comparison of their properties,

including their mass spectrometric cracking patterns, with those of authentic samples. A third component of the products, also isolated by gas chromatography, is believed to be 1 : 3-dichlorohexafluoropropane on the basis of its retention volume and mass spectrum, but unfortunately no authentic sample of this material was available to confirm this. Mass spectrometric examination of the reaction mixture has revealed, in addition to mass peaks characteristic of the ketone and of the compounds named above, small mass peaks which can only arise from 1 : 4-dichlorohexafluoromethyl ethyl ketone, and from 1 : 4-dichloro-octafluorobutane. These overall reaction products may be understood on the basis of the following reaction scheme :

$$\begin{aligned}
&ClCF_2COCF_2Cl \xrightarrow{h\nu} 2\ CF_2Cl— + CO\\
&CF_2Cl— + ClCF_2COCF_2Cl \rightarrow CF_2Cl_2 + ClCF_2COCF_2—\\
&CF_2Cl— + CF_2Cl— \rightarrow ClCF_2CF_2Cl\\
&CF_2Cl— + ClCF_2COCF_2 \rightarrow ClCF_2COCF_2CF_2Cl\\
&ClCF_2COCF_2CF_2Cl \xrightarrow{h\nu} CF_2Cl— + ClCF_2CF_2— + CO\\
&ClCF_2CF_2— + CF_2Cl— \rightarrow ClCF_2CF_2CF_2Cl\\
&ClCF_2CF_2— + ClCF_2CF_2— \rightarrow ClCF_2CF_2CF_2CF_2Cl
\end{aligned}$$

The excited states involved in the primary decomposition of the ketone may be quenched by the ketone and by other molecules. The behaviour of the ketone in this respect resembles closely that of hexafluoroacetone. A further reaction product that is to be anticipated from the free radicals involved in this system is 1 : 6 dichloro-octafluoro-hexa-2,5-dione, which may be formed by the combination of two chlorotetrafluoro-acetonyl radicals. Although no evidence for the formation of this compound has been obtained, this does not exclude the possibility that it occurs in the products of reaction, since its observation may be made difficult by its anticipated relatively low volatility.

The proportion of dichlorodifluoromethane produced in the photolysis increases with temperature, and further study of this effect will give the energy of activation of the abstraction process.

Where radicals of two species, R_1 and R_2, are involved in a reaction system these may combine in the following ways :

$$\begin{aligned}
R_1 + R_2 &\longrightarrow \text{products : rate constant } k_{12}\\
R_1 + R_1 &\longrightarrow \quad ,, \quad ,, \quad ,, \quad k_{11}\\
R_2 + R_2 &\longrightarrow \quad ,, \quad ,, \quad ,, \quad k_{22}
\end{aligned}$$

The quantity $\phi^2 = k^2_{12}/k_{11}k_{22}$ is a measure of the preference of unlike radicals to combine. For the chlorodifluoromethyl and 2-chlorotetrafluoroethyl radicals in the present system it has been possible to estimate the rates of accumulation of the ethane, propane and butane, which are formed by these processes, from the mass spectra of reaction mixtures photolysed to small extents. These give $\phi = 2{\cdot}2$, which is about the value required by the simple collision theory of reaction-rates, and is a value close to those tabulated recently for a number of free radicals[3].

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[1] Davis, W., *Chem. Revs.*, **40**, 201 (1947).
[2] Ayscough, P. B., and Steacie, E. W. R., *Proc. Roy. Soc.*, A, **234**, 476 (1956).
[3] Trotman-Dickinson, A. F., Ann. Rep. Chem. Soc., **55**, 39 (1959).

Identification of the Structure of Non-volatile Organic Substances by Gas Chromatography of Pyrolytic Products

At the Third International Symposium on Microchemistry in Birmingham, Prof. F. Feigl reported on the combination of coloured spot reactions with the pyrolysis of high molecular weight substances for identification purposes[1]. I wish to point out some new possibilities of this type of analysis in the study of the constitution of non-volatile or poorly volatile substances, combining pyrolytic reactions with gas chromatography.

Gas chromatography has been commonly used in the analysis of the products of pyrolytic processes. The work of Desty *et al.*[2] in following the function of internal combustion engines or of Kyryacos *et al.*[3] on the composition of the cool-flame products will be remembered. Recently, gas chromatography has been used for the analysis of substances by the examination of the functional groups split off, the determination of alkoxy-groups[4–6], for example. More recently, a short report on the analysis of pyrolytic products of some polymers[7,8] appeared in the literature.

The principle of the method proposed is very simple. The substance is submitted to controlled pyrolysis in a stream of carrier gas and the products produced are separated and identified by gas chromatography. It can be supposed that the composition of the pyrolytic products will bear a close relation to the structure of the original substance if the pyroly-

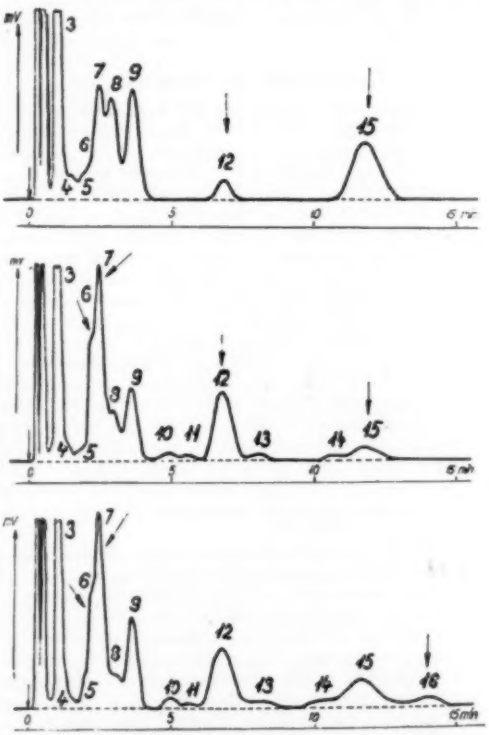

Fig. 1. Chromatograms of the pyrolytic products (C_1–C_5 hydrocarbons) of oils (olive, hardened olive and coconut oil). Fractions 1–4, C_1 + C_2–C_5 hydrocarbons, fractions 5–9, C_6 hydrocarbons, fractions 10–16, C_6 hydrocarbons (squalane, temperature, 40° C.)

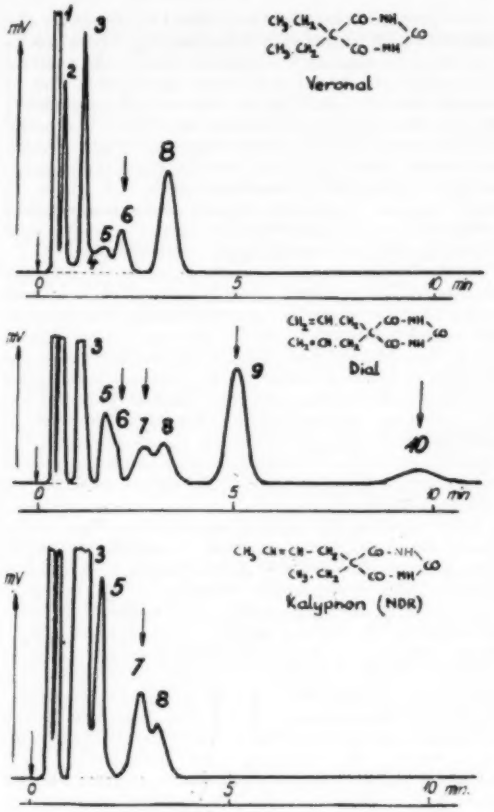

Fig. 2. Chromatograms of pyrolytic products in the area of C_2–C_6 hydrocarbon fragments of sodium salts of barbituric acid derivatives. Fraction 1, C_1 and C_2 hydrocarbons, fraction 2, C_3 hydrocarbons, fractions 3 and 4, C_4 hydrocarbons, fractions 5–8, C_5 hydrocarbons, fractions 9 and 10, C_6 hydrocarbons (squalane, temperature, 40° C.)

sis can be hindered from occurring as a complex reaction of the original substance and the decomposition products being formed. It is necessary in order to achieve a satisfactory solution to have sufficiently extensive measurements of the chromatographic spectra of the various decomposition products and to use an instrumentation technique which allows working with microgram quantities of substances.

Our experiments were made with a high-temperature chromatograph manufactured by the firm (National Plant) Laboratory Instruments, Prague, which is equipped with a flame ionization detector, circulation air thermostat operating up to 350° C., and a recorder with a range of 0·2 mV. Squalane (30 per cent w/w on 'Celite 545') was used as stationary phase, nitrogen as carrier gas, and hydrogen served to maintain the flame. The substance was put on to the column in a quantity up to 0·1 mgm. directly or from an ethereal solution, on a platinum wire of 0·1 mm. in diameter, shaped in spiral form, 15 mm. in length and 1·5 mm. in diameter. By means of an electric time-switch reproducible heating of the platinum wire (up to 800° C.) for a precisely adjusted time (0·1–5·0 sec.) can be attained.

Experiments made with various plant oils and animal fats showed a surprising reproducibility of the chromatographic spectrum quality as well as a quantitative representation of the components, even with relatively large variations of the platinum wire temperatures and the heating time. This situation occurs—and this must be emphasized—only on using minute quantities of substances, 10–50 μgm., when an instantaneous removal of the pyrolytic products from the decomposition area takes place. The pyrolysis of substances with relatively small molecules (for example, fats, steroids) tends to be an 'isothermal' action and the molecule fragments are the result of the power balance within the molecule at this decomposition point and the equilibrium of radicals produced. In the case of pyrolysis of macromolecules such as rubber, etc., the qualitative reproducibility of chromatographic spectrum remains similar, but the quantitative composition is not so consistent.

Fig. 1 shows the pyrolytic spectrum of the C_1–C_6 hydrocarbon products which are formed by the pyrolysis of olive, hydrogenated olive and coconut oils. An obvious qualitative and quantitative shifting of the components in the chromatograms with a change of the chemical nature of the oil is apparent. Other oils, such as linseed oil or castor oil, also exhibit qualitatively and quantitatively specific pyrolytic spectra.

In order to clarify the relation between the composition of the decomposition products and the structure of the original substance, the pyrolysis products of a series of simple structurally related barbituric acid derivatives were studied. In Fig. 2 the chromatograms of three of the fourteen barbiturates studied can be seen. Fragments of the molecules present are always qualitatively as well as quantitatively highly specific. Not only can the group type of a soporific be differentiated, but also its

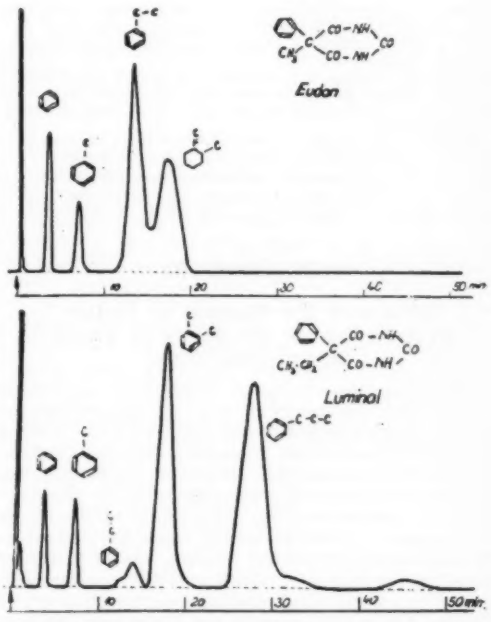

Fig. 3. Chromatogram of the pyrolytic products of the sodium salts of barbituric acid phenyl derivatives in the area of aromatic hydrocarbon fragments (squalane, temperature, 100° C.)

structure can be exactly determined. Experiments made *in vivo* supported the opinion that it would be possible to prove the structure of a barbiturate, after being taken, not only in the stomach content but also in the urine.

The pyrolytic procedure gives not only primary decomposition products. In the decomposition process radicals are formed which react in a characteristic way and form new components indicative of the structure of the original radicals. In Fig. 3 a spectrum of fragments in a range higher than benzene can be seen which shows components that are formed necessarily by the recombination of radicals.

A further improvement permits the use of various chromatographic column fillings for a double or multiple proof of the structure of the pyrolytic substances, the choice of various carrier gases for inducing structural changes in the decomposition products being formed and a temperature change of the chromatographic column (Figs. 2 and 3) for the exposure of a suitable part of the chromatographic spectrum.

This technique, particularly the gas chromatography, has considerably extended analytical potentialities in the field of the poorly volatile and non-volatile substances and is applicable to oils, alkaloids, steroids and other similar natural substances, artificial dyestuffs, drugs and other synthetic materials. Further, it serves as a basis for the development of rapid inquiry methods for medicinal and forensic chemistry, toxicology, biochemistry and biology, and is obviously useful in proving and verifying the structure of chemical individuals. Studies outlined in this communication will be published elsewhere in detail.

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[1] Feigl, F., Proc. Symp. Microchemistry, Birmingham, 1958 (Pergamon Press, London, 1959).
[2] Desty, D. H., Warman, T. J., and Whyman, B. H. F., Symp. Vapour Phase Chromatography, London, 1956, 346 (Butterworth, 1957).
[3] Kyryacos, G., Menapace, H. R., and Boord, C. F., *Anal. Chem.*, **31**, 2, 222 (1959).
[4] Vertalier, S., and Martin, F., *Chim. analytique*, **40**, 3, 80 (1958).
[5] Kratzl, K., and Grubner, K., *Monatschr.*, **89**, 45, 618 (1958).
[6] Springer, H., and Market, T., *Mikrochim. Acta*, 122 (1959).
[7] Lehrle, R. C., and Robb, J. C., *Nature*, **183**, 1671 (1959).
[8] Radell, E. A., and Strutz, H. C., *Anal. Chem.*, **31**, 1890 (1959).

BIOCHEMISTRY

Effects of the Method of Tissue Preparation on the Assay of Tissue Enzyme Activities

There has recently been considerable interest in comparative enzyme-levels of various tissues of different species in health and disease. It is evident that the quantitative determination of enzyme activity in tissues is dependent not only upon the method used for measuring the activity, but also upon the method used for preparation of the tissue for analysis. Various factors, such as the intracellular localization of the enzyme, the availability of coenzymes and activators, and the permeability of both cellular and particulate membranes of different tissues to various substrate and product molecules, play a part in the effect of the preparation of the tissue prior to enzyme assay.

As a general principle, the method of choice for the preparation of tissue should be that by which maximum enzyme activity is obtained, that is, the method of preparation should not cause appreciable loss of enzyme activity, and should remove all membranes limiting the rate of the enzyme reaction. The assay medium should have optimal composition and contain any known coenzymes and activators of the enzyme studied. If careful consideration is not given to these matters significant discrepancies may appear in the work of a single laboratory or in the comparison of results obtained by different investigators.

In the course of some recent studies on the activity of alkaline phosphatase in the kidney of a patient with hypophosphatasia[1], a considerable discrepancy was noted between the results obtained using frozen-dried 16μ sections[2] and those obtained with aqueous extracts of tissue minces[2–5]. In order to investigate the cause of the discrepancy, alkaline phosphatase activity was determined by Lowry's method[6] in rabbit kidney cortex and liver tissue prepared for analysis by various methods. Also determined was lactic dehydrogenase activity by means of a fluorometric method[7].

Five methods of sample preparation were used: frozen-dried sections, crushed frozen-dried tissue, homogenate, frozen-dried homogenate, and minced tissue. The homogenate technique was included because it is the most widely used method of tissue preparation for enzyme assays. The crushed frozen-dried tissue method and the frozen-dried homogenate technique were included because they offer the possibility of long-term storage of the preparations. To obtain frozen-dried sections the tissue was frozen in liquid nitrogen and sections 16μ thick were cut at −20° C. The sections were then frozen-dried, weighed, and placed directly in the substrate medium. For the crushed frozen-dried tissue, the tissue frozen in liquid nitrogen was crushed in a pre-cooled stainless steel mortar by a blow of a hammer on the pestle. Part of the powder was inserted into a previously weighed cold 13 mm. × 100 mm. 'Pyrex' tube, weighed, and lyophilized. The tube was then stoppered and stored at − 35° C. until used. Prior to analysis, the powder was reconstituted with enough water to make it 5 per cent on a wet-weight basis. For the homogenate a 5 per cent aqueous homogenate was made in a Potter–Elvehjem tissue grinder using a Kontes Glass Co. stainless steel pestle (clearance 0·009–0·011 in.) and then a Kel-*F* pestle (clearance 0·004–0·006 in.). The homogenization was continued long enough to pass all the tissue up and down a few times. To obtain frozen-dried homogenate, 1 ml. aliquots of the homogenate were shell frozen in 13 mm. × 100 mm. 'Pyrex' tubes and lyophilized. The tubes were stoppered and stored at − 35° C. until used. The powder was reconstituted with 1 ml. water prior to analysis. In the case of the mince, the tissue was finely minced with scissors and suspended in enough water to make it 5 per cent on a wet-weight basis. The suspension was covered with toluene and stored at 4° C.

The results are summarized in Table 1, upper part. Frozen-dried tissue sections showed both alkaline phosphatase activity and lactic dehydrogenase activity 2–8 times greater than any other preparation. The enzyme activity of frozen-dried homogenate in most instances showed somewhat greater activity than crushed frozen-dried tissue, homogenate or minced tissue. Homogenate and minced tissue stored

Table 1. Alkaline Phosphatase and Lactic Dehydrogenase in Liver and Kidney Cortex of Rabbit

Treatment	Alkaline phosphatase		Lactic dehydrogenase	
	Liver	Kidney cortex	Liver	Kidney cortex
Frozen-dried section	100 ± 3·5 (10)	100 ± 3·6 (8)	100 ± 4·8 (7)	100 ± 5·3 (8)
Frozen-dried homogenate	35 ± 1·4 (3)	33 ± 0·9 (3)	44 ± 1·4 (3)	37 ± 2·0 (3)
Crushed frozen-dried	18 ± 1·6 (2)	—	38 ± 4·8 (2)	—
Homogenate, 1*d*, −35° C.	17 ± 0·6 (2)	29 ± 0·9 (2)	29 ± 0·8 (2)	32 ± 1·4 (2)
,, 7–8*d*, −35° C.	18 ± 0·8 (2)	29 ± 2·3 (2)	27 ± 3·7 (2)	30 ± 3·5 (2)
,, 7–8*d*, +4° C.	28 ± 1·0 (2)	45 ± 1·8 (2)	24 ± 4·9 (2)	26 ± 2·6 (2)
Mince, 1*d*, + 4° C.	20 ± 0·6 (3)	33 ± 1·9 (3)	25 ± 0·4 (3)	32 ± 1·6 (3)
,, 7–8*d*, + 4° C.	35 ± 1·6 (3)	36 ± 1·8 (3)	12 ± 1·7 (3)	25 ± 2·2 (3)
Fresh homogenate: in water	100 ± 2·7 (3)	100 ± 3·2 (3)	100 ± 5·0 (3)	100 ± 4·2 (3)
in PO_4—BSA*	83 ± 3·4 (3)	60 ± 3·7 (3)	116 ± 2·9 (3)	90 ± 1·4 (3)
Frozen homogenate, 1*d*, −35° C. in water	96 ± 5·6 (3)	113 ± 4·5 (3)	112 ± 2·5 (3)	99 ± 1·8 (3)
in PO_4—BSA*	75 ± 1·3 (3)	62 ± 2·3 (3)	99 ± 3·4 (3)	83 ± 5·8 (3)

* 0·1 *M* phosphate buffer, *p*H 7·4, containing 0·05 per cent bovine serum albumin.

All activities based on dry weight; activities in upper part of table expressed in percentage of the activity of a frozen-dried section of the same tissue, in lower part in percentage of the activity of the fresh homogenate in water. Standard errors of the means shown with number of samples in parentheses.

for 7–8 days showed slightly greater alkaline phosphatase activity and slightly lower lactic dehydrogenase activity than homogenate and minced tissue stored for only one day. In the case of alkaline phosphatase, a very stable enzyme, the rate of solubilization of the enzyme into the extracting fluid is apparently higher than the rate of denaturation, while in the case of lactic dehydrogenase, a less stable enzyme, the rate of denaturation is apparently faster than the rate of solubilization. The high results obtained with frozen-dried sections can probably be explained by the rapid and complete rupture of cell membranes which occurs when the section is put into contact with aqueous substrate. Microscopically this process has been observed as a very rapid swelling and soaking of the dry tissue at the moment of immersion.

In another experiment homogenization was carried out in 0·1 *M* phosphate buffer (*p*H 7·4), containing 0·05 per cent bovine serum albumin, instead of in water. In the same experiment fresh homogenates were compared with those kept at − 35° C. for one day. The results are given in Table 1, lower part. No increase in the activity of either enzyme was noticed by replacing water with the phosphate–albumin medium. In the case of alkaline phosphatase a slight inhibition occurred, probably due to a mass law effect. No significant differences existed between the fresh homogenates and the homogenate which had been frozen and kept at − 35° C. for one day.

It appears that the highest results for both enzymes were found in the frozen-dried sections, while the frozen-dried homogenate method seems to be the next best technique. It is probable that differences in enzyme activity, depending on the method of preparation of tissues for analysis, will also be found for other enzymes. It appears evident that certain discrepancies in comparative tissue enzyme studies can be avoided if cognizance is taken of the critical importance of the methods of preparation of tissues for enzyme assay and the need for careful standardization in each study.

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[1] Rosenthal, I. M., Bonting, S. L., Hogan, W., and Pirani, C. L., *A. Med. Assoc. J. Dis. Child.* (in the press).
[2] Bonting, S. L., Pollak, V. E., Muehrcke, R. C., and Kark, R. M., *Science*, **127**, 1342 (1958).
[3] Bodansky, A., *J. Biol. Chem.*, **101**, 93 (1933).
[4] Woodward, H. Q., *Cancer Res.*, **2**, 497 (1942).
[5] Woodward, H. Q., *Cancer*, **9**, 352 (1956).
[6] Lowry, O. H., Roberts, N. R., Wu, M. L., Hixon, W. S., and Crawford, E. J., *J. Biol. Chem.*, **207**, 19 (1954).
[7] Lowry, O. H., Roberts, N. R., and Kapphahn, J. T., *J. Biol. Chem.*, **224**, 1047 (1957).

Formation of Carotene *in vitro*

Results presented in this communication indicate that both β-hydroxy β-methylglutarate and mevalonate may be considered precursors of carotene formation in an *in vitro* extract obtained from mycelial mats of the mould *Phycomyces blakesleeanus*. This extract, capable of synthesizing carotene, can also be used for establishing the existence of a back reaction from the mevalonate to β-hydroxy β-methylglutarate, for brevity referred to as glutarate.

The sequence of conversions is, however, undoubtedly similar to those previously proposed for carotenoids[1–4] and sterols[5]. In the initial stages of the formation of isoprenoid compounds, at least, there is an identity in the 5 and 6 carbon intermediates.

Previous work has shown[6] that glutarate and mevalonate are used with considerable efficiency in the synthesis of carotenoids in tomatoes, tomato and carrot homogenates, and by growing cultures of *Phycomyces*[1–4]. Goodwin has reviewed evidence that glutarate is a direct precursor of mevalonate in plant systems[3]. However, the experimental conditions in growing plants were such that enzymatic breakdown to acetate could occur, and thus mevalonate might arise independently.

In a series of tests, a cell-free extract has been obtained from mycelial mats of *Phycomyces* which is capable of synthesizing labelled carotene from acetate 2-^{14}C, glutarate 3-^{14}C and mevalonate 2-^{14}C.

The soluble system was isolated from five-day old cultures of *Phycomyces* grown on a medium containing 2·5 per cent glucose, 0·125 per cent leucine, 0·125 per cent asparagine, with added salts and thiamine. The extract was prepared by disintegrating the mycelial mats (about 100 gm. fresh weight) in a loose-fitting tissue homogenizer, at *p*H 7·8 in 0·2 *M* *tris*–hydrochloric acid buffer which contained in addition nicotinamide, versene, and glutathione,

all 0·002 *M*. The homogenate was centrifuged at 4,000*g* for 10 min. to remove gross particles, after which the supernatant was decanted. This was dialysed against 0·002 *M* *tris*–hydrochloric acid buffer for 12 hr. All manipulations were carried out at 4° C. The dialysed material contained 11 mgm. of protein per ml. In an experiment, 4 ml. aliquots were placed in flasks, containing 10 μM of adenosine triphosphate, 1 μM each of diphosphopyridine nucleotide, triphosphopyridine nucleotide and reduced diphosphopyridine nucleotide and 10 μM magnesium sulphate. Substrates in the amount of 5 μM per 4 ml. were added, and the mixture was then incubated for 18–30 hr. at room temperature.

A protein pellet containing the carotene was obtained by centrifuging the test mixture at 10,000*g*. The β-carotene was extracted from the pellet, chromatographed, and the carotene was crystallized with the aid of carrier, washed with hot absolute alcohol and dried as previously described[4]. The carotene was then dissolved in toluene and bleached under a light source rich in ultra-violet for 8 hr. in capped vials. After bleaching, scintillation phosphors ('PPO' and 'popo') were added by injection through the rubber cap of the vial and the resultant solution counted in a Packard 'Tricarb' scintillation counter.

The interconversion of substrates in the supernatant was established as follows. The solution was passed over a 'Dowex 50' column and afterwards over one of 'Dowex 1', on which the free organic acids were absorbed. They were eluted, re-passed over 'Dowex 50', and thus isolated. Phosphorylated derivatives are apparently lost by this procedure, and only the free acids were obtained. The solution was then concentrated and portions were chromatographed on Whatman No. 1 paper, by use of ethanol–ammonia–water (80 : 5 : 15), solvent 1, and *n*-butanol–acetic acid–water (4 : 1 : 5), solvent 2. The paper was then sprayed with bromphenol blue. The activity of the spots was determined by removing strips of the paper containing the visualized spots and counting in sections in a windowless flow counter.

Table 1 lists the total counts added in the different substrates used, and the resultant activities found in the β-carotene. The activity of the substrates was determined by scintillation counting and the counts were adjusted against an internal counting standard to place results on a comparable basis.

For mevalonate, between 1 and 4 per cent of the activity was incorporated into the carotene; for glutarate, about 0·7 per cent; for acetate 2-^{14}C, only a trace. These figures represent lower limits for recovery of activity since the carotene was crystallized.

Table 2 lists the activity of the organic acids isolated from the supernatants. Acetate gives rise to both mevalonate and glutarate, and these are interconvertible. No significance can be attached to the ratios of glutarate : mevalonate activity as there is no information as to the extent to which glutarate may have been diverted into other paths. In addition to well-defined spots for glutarate and mevalonate with R_F values (inadvertently reversed in our earlier paper[4]) of 0·65, 0·59, respectively, for solvent 1, and 0·80, 0·78 for solvent 2, there was an unknown substance showing considerable activity with R_F values of 0·39 and 0·76 in the two solvents.

Table 1. Incorporation of Activity into β-Carotene

Substrate	Total c.p.m. added	Total c.p.m. in carotene
Acetate 2-^{14}C	1,100,000	265
Mevalonate 2-^{14}C	170,000	6,997
Mevalonate 2-^{14}C	370,000	3,724
Glutarate 3-^{14}C	187,000	1,296

Table 2. Interconversion of Organic Acids

Organic acids substrate added	Total c.p.m. found Mevalonate	Glutarate
Acetate 2-^{14}C	202	1,226
Mevalonate 2-^{14}C	(18,170)	4,140
Glutarate 3-^{14}C	2,096	(2,392)

The net increase in carotene in these experiments is usually of the order of 5–10 μgm. The mildest treatment in the homogenizer with removal of unbroken cells by centrifugation seems the most promising lead to improvement of yield.

The foregoing results indicate that both glutarate and mevalonate may be considered as precursors of carotenoids in the system studied, and the evidence substantiates existence of a back reaction from mevalonate to the (hydroxy- methyl-) glutarate.

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[1] Purcell, A. E., Thompson, G. A., and Bonner, J., *J. Biol. Chem.*, **234**, 1081 (1959).
[2] Shneour, E. A., and Zabin, I., *J. Biol. Chem.*, **234**, 770 (1954).
[3] Goodwin, T. W., "Adv. in Enzymol.", **21**, 295 (1959).
[4] Chichester, C. O., Yokoyama, H., Nakayama, T. O. M., Lukton, A., and Mackinney, G., *J. Biol. Chem.*, **234**, 598 (1959).
[5] Lynen, F., in "Biosynthesis of Terpenes and Sterols", ed. by Wolstenholme, G. E. W., and O'Connor, M. (Churchill, Ltd., London, 1959).
[6] Chichester, C. O., Nakayama, T. O. M., and Mackinney, G., *J. Biol. Chem.*, **214**, 515 (1955).

Influence of Sorbic Acid on Acetate Oxidation by *Saccharomyces cerevisiae* var. *ellipsoideus*

Sorbic acid (2,4-hexadienoic acid) is widely used as a food preservative. Its effective action against different micro-organisms[1–5] and its harmlessness as a dietary component[6], as well as the ease of its quantitative determination[7–9], and its chemical stability[9] are among the main reasons for its rapid adoption in the food industry.

Sorbic acid has a selective action against catalase-positive organisms[10], and its use as a component of enrichment media for species of *Clostridium* has been proposed[11]. Although the mechanism of its inhibitory action is not well understood, it has been suggested that it probably interferes with the activity of the dehydrogenases in the cell[12].

In the experiments to be described in this communication, we have used a strain of *Saccharomyces cerevisiae* var. *ellipsoideus* which was isolated from fermenting grape juice. The cells were grown on autolysed yeast–dextrose agar. After incubation for some 15 hr. at 30° C., they were harvested and washed twice with 0·033 *M* monopotassium phosphate solution, and finally resuspended in the same solution to an optical density corresponding to a concentration of 2·0 mgm. dry matter per ml. 2 ml. of this suspension were used for each Warburg

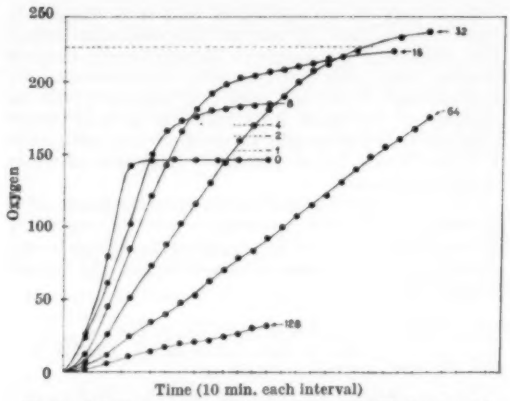

Fig. 1. 4·0 mgm. (dry weight) of cells in each Warburg vessel. Potassium hydroxide in the central cup. 5 μmol. of acetate in all cases. The total amount of sorbate (in μmol.) is indicated at the end of each curve. The upper dotted line represents the theoretical amount of oxygen required for complete oxidation of acetate

vessel, and the oxygen uptake was measured at 30° C. with the conventional technique. Our first experiments showed that there was a small but definite increase of the oxygen uptake over the endogenous by cells in the presence of this compound. This effect was found to be due to an impurity which could be removed by recrystallization from water.

Fig. 1 shows the effect of different concentrations of sorbic acid (added as the sodium salt) on the oxidation of acetate by our strain. The compound has an influence on both the respiration-rate and on the total oxygen uptake by the cells. In the cases where 1, 2 and 4 μmol. of sorbate had been used, only the final values are indicated in the graph. Carbon dioxide determinations by the indirect method showed a respiratory quotient of 1 in all cases.

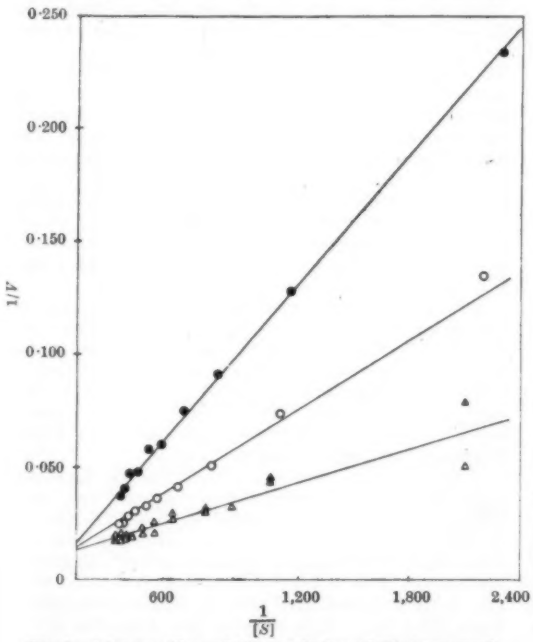

Fig. 2. 4·0 mgm. (dry weight) of cells in each Warburg vessel. Potassium hydroxide in the central cup. The concentration of acetate was varied from 1 to 10 μmol. The straight part of each curve was used for calculating the velocities of respiration. △, No inhibitor; ○, 16 μmol. sodium sorbate; ●, 32 μmol. sodium sorbate

The inhibitory effect of sorbate on the respiration-rate is competitive, as shown by a 'double reciprocal' plot (Fig. 2). Although the exact mechanism of the competition has not been determined, some preliminary experiments seem to indicate that sorbate interferes with the synthesis of citric acid in the cells, probably by way of formation of sorbyl–coenzyme *A*. Sorbic acid also inhibits the acetylation of sulphanilamide by pigeon liver extracts.

The inhibition of acetate assimilation is more difficult to explain. There was no appreciable effect of sorbate on the oxidative phosphorylation by whole cells in the presence of acetate and the inhibitor. Since our manometric experiments were performed under conditions (absence of carbon dioxide in the atmosphere and of combined nitrogen in the medium) which rule out any significant assimilation through the Krebs cycle, it is possible that sorbate could interact with the synthesis of higher fatty acids by the resting cells.

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[3] Costilow, R. N., Ferguson, W. E., and Ray, S., *App. Microbiol.*, **3**, 341 (1955).
[4] Costilow, R. N., Coughlin, F. M., Robbins, E. K., and Hsu, Wen-Tah, *App. Microbiol.*, **5**, 373 (1957).
[5] Bell, T. A., Etchells, J. L., and Borg, A. F., *J. Bact.*, **77**, 573 (1959).
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In the course of an investigation into the nature of the soluble carbohydrate materials released by phytoplankton into their culture medium[1], we found that media from cultures of *Amphidinium carteri*, a dinoflagellate, showed a distinctly blue reaction with N-ethyl carbazole, rather than the usual purple-red colour. The material causing the blue reaction could be removed by dialysis, which also removed the fishy odour characteristic of old cultures of this organism. The dialysate displayed the usual carbohydrate colour reaction.

The substance responsible for the blue reaction, and presumably also for the fishy odour, is very labile. The product originally released into the medium by this organism is somewhat more complex than the dialysable substance responsible for the blue reaction, since samples of filtered medium taken from bacteria-free cultures in the logarithmic stage of

all 0·002 *M*. The homogenate was centrifuged at 4,000*g* for 10 min. to remove gross particles, after which the supernatant was decanted. This was dialysed against 0·002 *M* *tris*-hydrochloric acid buffer for 12 hr. All manipulations were carried out at 4° C. The dialysed material contained 11 mgm. of protein per ml. In an experiment, 4 ml. aliquots were placed in flasks, containing 10 μM of adenosine triphosphate, 1 μM each of diphosphopyridine nucleotide, triphosphopyridine nucleotide and reduced diphosphopyridine nucleotide and 10 μM magnesium sulphate. Substrates in the amount of 5 μM per 4 ml. were added, and the mixture was then incubated for 18–30 hr. at room temperature.

A protein pellet containing the carotene was obtained by centrifuging the test mixture at 10,000*g*. The β-carotene was extracted from the pellet, chromatographed, and the carotene was crystallized with the aid of carrier, washed with hot absolute alcohol and dried as previously described[6]. The carotene was then dissolved in toluene and bleached under a light source rich in ultra-violet for 8 hr. in capped vials. After bleaching, scintillation phosphors ('PPO' and 'popo') were added by injection through the rubber cap of the vial and the resultant solution counted in a Packard 'Tricarb' scintillation counter.

The interconversion of substrates in the supernatant was established as follows. The solution was passed over a 'Dowex 50' column and afterwards over one of 'Dowex 1', on which the free organic acids were absorbed. They were eluted, re-passed over 'Dowex 50', and thus isolated. Phosphorylated derivatives are apparently lost by this procedure, and only the free acids were obtained. The solution was then concentrated and portions were chromatographed on Whatman No. 1 paper, by use of ethanol–ammonia–water (80 : 5 : 15), solvent 1, and *n*-butanol–acetic acid–water (4 : 1 : 5), solvent 2. The paper was then sprayed with bromphenol blue. The activity of the spots was determined by removing strips of the paper containing the visualized spots and counting in sections in a windowless flow counter.

Table 1 lists the total counts added in the different substrates used, and the resultant activities found in the β-carotene. The activity of the substrates was determined by scintillation counting and the counts were adjusted against an internal counting standard to place results on a comparable basis.

For mevalonate, between 1 and 4 per cent of the activity was incorporated into the carotene; for glutarate, about 0·7 per cent; for acetate 2-^{14}C, only a trace. These figures represent lower limits for recovery of activity since the carotene was crystallized.

Table 2 lists the activity of the organic acids isolated from the supernatants. Acetate gives rise to both mevalonate and glutarate, and these are interconvertible. No significance can be attached to the ratios of glutarate : mevalonate activity as there is no information as to the extent to which glutarate may have been diverted into other paths. In addition to well-defined spots for glutarate and mevalonate with R_F values (inadvertently reversed in our earlier paper[4]) of 0·65, 0·59, respectively, for solvent 1, and 0·80, 0·78 for solvent 2, there was an unknown substance showing considerable activity with R_F values of 0·39 and 0·76 in the two solvents.

Table 1. Incorporation of Activity into β-Carotene

Substrate	Total c.p.m. added	Total c.p.m. in carotene
Acetate 2-^{14}C	1,100,000	265
Mevalonate 2-^{14}C	170,000	6,997
Mevalonate 2-^{14}C	370,000	3,724
Glutarate 3-^{14}C	187,000	1,296

Table 2. Interconversion of Organic Acids

Organic acids substrate added	Total c.p.m. found Mevalonate	Glutarate
Acetate 2-^{14}C	202	1,226
Mevalonate 2-^{14}C	(18,170)	4,140
Glutarate 3-^{14}C	2,096	(2,392)

The net increase in carotene in these experiments is usually of the order of 5–10 μgm. The mildest treatment in the homogenizer with removal of unbroken cells by centrifugation seems the most promising lead to improvement of yield.

The foregoing results indicate that both glutarate and mevalonate may be considered as precursors of carotenoids in the system studied, and the evidence substantiates existence of a back reaction from mevalonate to the (hydroxy- methyl-) glutarate.

Acknowledgment is made to the National Institutes of Health for a grant-in-aid to C. O. Chichester, and to the National Science Foundation for a grant-in-aid to G. Mackinney.

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[1] Purcell, A. E., Thompson, G. A., and Bonner, J., *J. Biol. Chem.*, **234**, 1081 (1959).
[2] Shneour, E. A., and Zabin, I., *J. Biol. Chem.*, **234**, 770 (1954).
[3] Goodwin, T. W., "Adv. in Enzymol.", **22**, 295 (1959).
[4] Chichester, C. O., Yokoyama, H., Nakayama, T. O. M., Lukton, A., and Mackinney, G., *J. Biol. Chem.*, **234**, 598 (1959).
[5] Lynen, F., in "Biosynthesis of Terpenes and Sterols", ed. by Wolstenholme, G. E. W., and O'Connor, M. (Churchill, Ltd., London, 1959).
[6] Chichester, C. O., Nakayama, T. O. M., and Mackinney, G., *J. Biol. Chem.*, **214**, 515 (1955).

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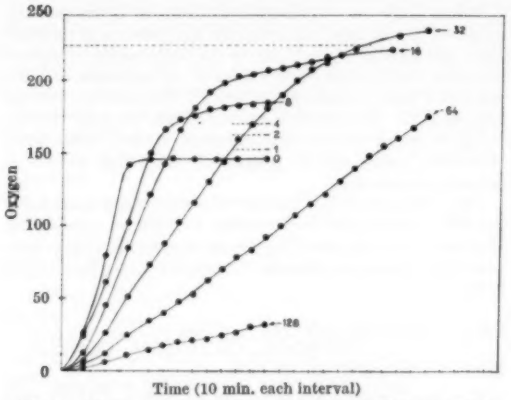

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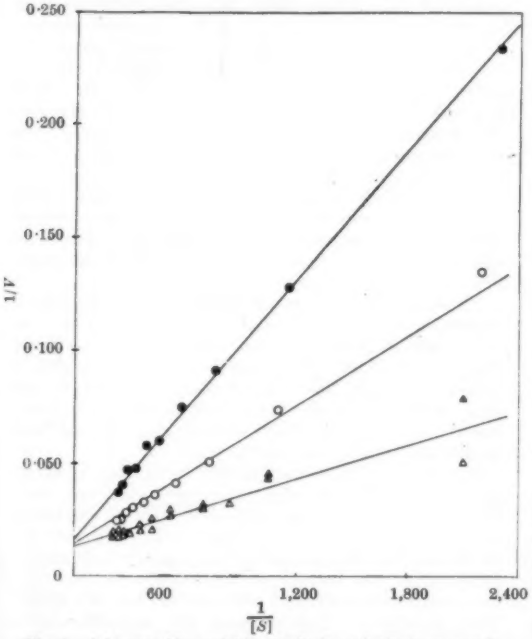

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The substance responsible for the blue reaction, and presumably also for the fishy odour, is very labile. The product originally released into the medium by this organism is somewhat more complex than the dialysable substance responsible for the blue reaction, since samples of filtered medium taken from bacteria-free cultures in the logarithmic stage of

growth do not develop the characteristic odour for several hours. The autohydrolysis of the parent compound can be prevented by making the medium slightly acid after filtration, and storing the material at low temperature.

The process of autohydrolysis can be followed by applying the cupric chloride test for aliphatic amines described by Hershenson and Hume[2]. If cold, slightly acid medium is tested, no immediate reaction is visible. After several hours at room temperature a definite positive colour reaction develops. If the parent substance is hydrolysed by refluxing with dilute sodium hydroxide, the test shows an immediate positive reaction.

The dinoflagellate cells contain a substance, probably an enzyme, which can hydrolyse the parent compound very quickly. If cells are damaged in the course of filtration, the fishy odour develops immediately. This hydrolysis can be prevented by the addition to the medium of eserine, an anticholinesterase. The end-product, however, displays none of the chemical reactions of choline. The original product may be an analogue of acetylcholine, since both acetylcholine and acetyl-β-methylcholine behave in the same manner as does the original product in the amine and N-ethyl carbazole tests.

It seems probable that *Amphidinium carteri* releases some analogue of acetylcholine either as a waste product or as a protective device. Such a compound should be physiologically active, and might act as effective protection against zooplankton. The organism itself contains a mechanism for breaking down this compound. This compound or similar compounds may be released by other dinoflagellates. Abbott and Ballantine[3] extracted centrifuged cells of *Gymnodinium veneficum* with acidified methanol and removed a compound the effects of which on mice were similar to those of acetylcholine. An acidified methanol extract of an activated carbon column through which medium from the *Gymnodinium* culture had been passed gave similar results.

This work was carried out at the Osborn Zoological Laboratory, Yale University, New Haven, Connecticut, and the U.S. Fish and Wildlife Laboratory, Milford, Connecticut.

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[1] Guillard, R. R. L., and Wangersky, P. J., *Limnol. and Oceanogr.*, **3**, 449 (1958).
[2] Hershenson, H. M., and Hume, D. N., *Anal. Chem.*, **29**, 16 (1957).
[3] Abbott, B. C., and Ballantine, D., *J. Mar. Biol. Assoc. U.K.*, **36**, 169 (1957).

Enzymic Synthesis of L-Aspartate-4-^{14}C

As the use of radioactive isotopes in research has been extended, there has been an increasing demand for specifically labelled metabolites of high activity. At present the synthesis of such compounds by purely chemical means is often difficult, if not impossible, particularly when only the biologically active isomer is required. While enzymic synthesis is recognized to be desirable in principle, some workers may be deterred by the difficulties involved in preparing the required enzymes to what is considered to be the necessary state of purity.

What may be overlooked is that it is frequently possible to isolate an enzyme biochemically rather than physically by adding to an adequately dialysed mixture only that combination of substrates, co-enzymes and co-factors which is necessary for its activation. In these circumstances it may prove possible, as in the present instance, to use crude (and therefore more easily prepared) enzymes to effect specific syntheses.

Previous work[1] has indicated that L-aspartate-4-^{14}C can be synthesized enzymically *in vitro* by combining the reactions catalysed by phosphopyruvate carboxylase[2] and glutamic–aspartic transaminase. The overall reaction is:

$$CH_2:COPO(OH)_2\cdot COOH + {}^{14}CO_2 + H_2O + HOOC\cdot CH_2\cdot CH_2\cdot CHNH_2\cdot COOH \rightarrow HOO^{14}C\cdot CH_2\cdot CHNH_2\cdot COOH + HOOC\cdot CH_2\cdot CH_2\cdot CO\cdot COOH + H_3PO_4 \quad (1)$$

Recently, experiments have been undertaken in this laboratory in order to determine whether this accurately represents the reaction involved.

The enzymes required for the synthesis were prepared by grinding 100 gm. of *Kalanchoë crenata* leaves in 100 ml. of 0·625 per cent 2-amino-2-hydroxymethylpropane-1 : 3-diol (*tris*). The protein present in the clear supernatant after centrifugation at 20,000*g* was precipitated by making 100 per cent w/v with ammonium sulphate. The precipitate was redissolved in 30 ml. of 1 per cent *tris* buffer at *p*H 7·5 and dialysed against 3 l. of this solution in the cold for 48 hr. and then against 3 l. of mineral-free water for a further 24 hr.

The reaction was started by the addition of excess phosphoenolpyruvate (50 μmoles) to a stoppered vessel of 7 ml. capacity containing sodium bicarbonate (approximately 22 μmoles at 22·8 μc./μmole), enzyme (5·0 ml.), magnesium chloride (7 μmoles), glutamate (75 μmoles), sodium phosphate (7 μmoles) and *tris* buffer (0·10 ml., 20 per cent w/v at *p*H 7·4). After incubation for 1 hr. at room temperature the reaction was stopped by the addition of formic acid. The acidified reaction mixture was boiled to remove formic acid, and then passed through a column of acid alumina which absorbed all the residual radioactivity. Water was passed through the column until activity appeared in the effluent. Ammonia was then passed through the column until the major part of the activity had been recovered.

Two-dimensional chromatography of the ammonium hydroxide eluate followed by radioautography indicated that radioactivity was restricted to two spots. The first of these corresponded in position to that of added aspartate and contained more than 99 per cent of the activity. The second spot, containing less than 1 per cent of the activity, has not yet been identified but may be oxaloacetate.

When the radioactive eluate from the aspartate spot on two-dimensional chromatograms was incubated with indanetrione hydrate in 'Methyl cellosolve' at *p*H 5 the activity was lost, indicating that the label was restricted to the carboxyl groups[3]. Similarly, the activity was lost as labelled carbon dioxide when the eluate was incubated with an enzyme preparation from *Nocardia*. This enzyme has been shown[4] to catalyse, specifically, the quantitative β-decarboxylation of L-aspartate and this result therefore provides convincing evidence that the labelled substance is in fact L-aspartate-4-^{14}C.

A comparison of the radioactivity of the aspartate fraction and that of standard carbonate-^{14}C (measured with a Geiger–Müller counter under similar conditions) indicated that the level of activity in the aspartate was greater than 400 μc. The amount of aspartate synthesized was determined by spectrophotometric measurements of reduced diphosphopyridine nucleotide oxidized in the overall enzymic reaction:

aspartate + α-oxoglutarate + $DPNH_2$ ⟶ malate + DPN + glutamate

and found to be approximately 20 μmoles. On the basis of this figure the specific activity of the aspartate was evidently close to that of the bicarbonate used in its synthesis.

These results clearly indicate that virtually all the carbon dioxide fixed is located in the β-carboxyl group of L-aspartate and are consistent with the view that equation 1 is an accurate representation of the reaction.

We wish to acknowledge a gift of *Nocardia* enzyme from Dr. Kerridge, M.R.C. Unit of Microbiology, University of Cambridge, and grants from the University of London Central Research Fund and the Department of Scientific and Industrial Research made to one of us (D. A. W.).

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[1] Walker, D. A., and Ranson, S. L., *Plant Physiol.*, **33**, 226 (1958).
[2] Bandurski, R. S., and Greiner, C. M., *J. Biol. Chem.*, **204**, 781 (1953).
[3] Nelson, C. D., and Krotkov, G., *Canad. J. Bot.*, **34**, 425 (1956).
[4] Crawford, L. V., *Biochem. J.*, **68**, 221 (1958).

Decrease in Coenzyme I Content of Spleen after Freezing

During an investigation into the effect of the level of coenzyme I on the response of tissues to hydrocortisone, it was necessary to keep the tissues for at least a day before the coenzyme-level could be estimated. As a supply of solid carbon dioxide ('Drikold') was available it was used to freeze the tissues quickly before storing in a deep freeze.

Table 1. Comparison of Diphosphopyridine Nucleotide (DPN^+) Content of Freshly Killed and Frozen Rat Tissues

Tissue	γ-DPN^+/gm. fresh weight ± *S.E.*				Percentage decrease in DPN^+ caused by freezing
	Freshly killed	No. of estimations	Frozen	No. of estimations	
Diaphragm	256 ± 13·25	4	250 ± 7·14	4	—
Kidney	275 ± 8·75	5	277 ± 11·84	4	—
Liver	236 ± 8·43	8	194 ± 6·12	7	18
Blood*	44·2 ± 2·35	5	36·3 ± 2·16	5	18
Spleen	129 ± 5·19	10	16·8 ± 1·41	12	87

* Blood results expressed as γ-DPN^+/ml. whole blood.

The method of extracting the oxidized coenzyme I was that of Glock and McLean[1], except that sodium carbonate was used for neutralizing the acid. The diphosphopyridine nucleotide was then estimated by the fluorescence method[2]. Approximately 100 mgm. of tissue was used for each estimation. Rat tissues investigated were diaphragm, kidney, liver and spleen. It was found that freezing as soon as the tissue was removed from the animal had little or no effect on the content of diphosphopyridine nucleotide except in the case of spleen. As spleen contains a high proportion of blood, this tissue was also investigated (Table 1).

Spleens from two other species—rabbit and guinea pig—were similarly treated and the same type of result obtained from frozen and unfrozen tissue (Table 2). Other available freezing agents—liquid oxygen and alcohol/solid carbon dioxide mixture—were used with rat spleen. Values of 17·8 and 34·0 γ-diphosphopyridine nucleotide per gm. of tissue were obtained respectively.

The buffer method for extracting coenzyme I from tissues of Jedeikin and Weinhouse[3] was tried with rat spleen and the fluorescence method used for the estimation. The average value for unfrozen tissue was higher than that obtained from the other extraction technique due to lower fluorescent blanks. Again, frozen tissues gave low results, the percentage of lowering being similar to that previously obtained (Table 2). When the complete method of Jedeikin and Weinhouse was used with large amounts of rat spleen (600 mgm.), diphosphopyridine nucleotide in extracts from frozen tissue was too small to measure accurately (Table 2).

Thawing frozen tissue before adding to the hot extracting fluid did not increase the value of coenzyme estimated above that of unthawed tissue. Length of freezing did not appear to affect the low results. Tissue which had been quickly frozen and estimated immediately gave results similar to tissues that had been stored frozen for some time. Freezing the neutralized extract from unfrozen spleen in solid carbon dioxide before fluorescence estimation did not lower the coenzyme content.

At first an inhibitor of the fluorescence was thought to be present. The blank obtained from frozen tissues was only 37 per cent of that from unfrozen tissues of the same weight, but known amounts of oxidized diphosphopyridine nucleotide (Sigma grade) added to the extracts or to the extracting acid at the same time as the tissues gave similar recoveries from both fresh and frozen tissues.

It was noticed that the colour of the neutralized extract from frozen tissue was a much darker brown than that from unfrozen tissue. Chromatography,

Table 2. Diphosphopyridine Nucleotide (DPN^+) Content of Fresh and Frozen Spleen Tissue from Different Species

Animal	Method of extraction*	Method of estimation*	γ-DPN^+/gm. fresh weight ± *S.E.* Fresh	No. of estimations	Frozen	No. of estimations	Percentage decrease in DPN^+ caused by freezing
Rabbit	1	2	156	2	12·6 ± 1·26	3	92
Guinea pig	1	2	112 ± 3·13	3	13·6 ± 1·18	3	88
Rat	1	2	129 ± 5·19	10	16·8 ± 1·41	12	87
Rat	3	2	159 ± 4·37	6	20·4 ± 1·39	4	87
Rat	3	3	119	2	—	2	—

* Figures relate to references as given in the text.

using pyridine/water as solvent[4], of these extracts after concentration by freeze drying showed larger brown non-fluorescent streaks in samples from frozen tissue than from fresh tissue, but no clearly defined diphosphopyridine nucleotide spot was detected.

The loss of coenzyme I content on freezing would not appear to be due to enzyme activity as the time for any breakdown is too short and the temperature too low. It also does not appear to be a simple inhibition in the fluorescence estimation. Thus apparently during freezing some physico-chemical phenomenon takes place.

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[1] Glock, G. E., and McLean, P., *Biochem. J.*, **61**, 381 (1955).
[2] Ciotti, M. M., and Kaplan, N. O., in "Methods in Enzymology", ed. by Colowick, S. P., and Kaplan, N. O., **3**, 896 (Academic Press, Inc., New York, 1957).
[3] Jedeikin, L. A., and Weinhouse, S., *J. Biol. Chem.*, **213**, 271 (1955).
[4] Burton, R. M., and San Pietro, A., *Arch. Biochem. Biophys.*, **48**, 184 (1954).

ANIMAL PHYSIOLOGY

Water Content of Cod (*Gadus callarias* L.) Muscle

In fatty fish such as herring the protein content of the flesh does not show any regular cycle of changes throughout the year[1,2]. Marked variation is shown by the fat, however, which decreases in times of food scarcity, the flesh showing a corresponding increase in water content. Non-fatty fish such as cod do not behave in this way, and in times of scarcity they draw on their body proteins both for metabolic purposes and for building up the gonads[3]. Water then takes the place of the protein, and in the case of cod artificially starved to the point of death it can increase to as much as 88 per cent of the flesh as compared with about 80·5 per cent in normal fish[4].

Although many determinations of the water content of fish muscle have been carried out, the rather small variations encountered in non-fatty fish landed at commercial ports have rarely been commented on. The following results show, however, that considerable biological information can be obtained from them.

The cod analysed were all obtained from the same ground, about 30 miles south-east of Aberdeen. They were filleted within 12 hr. of being caught, and myotomes 8–16, approximately (counting from the anterior end of the fillet) were dissected out free from myocommata (connective tissue) and placed in weighed basins, approximately equal weights of material being taken from each fish in a sample. Water was determined by drying in an oven at 100° C. for seven days.

Three size-groups of fish were used: (1) 20 in. long or less (sexually immature); (2) close to 30 in. long (about the size when they become mature); (3) 36–42 in. long (mature). It was difficult to get fish in the latter group at certain times of the year, and each sample consisted of pooled material from only 1–8 such fish. Material from medium-sized fish was obtained from 10 individuals, while 20 individuals were used for the 'small' group.

The results (Fig. 1) show that the water content is affected by the size of the fish as well as by the season, the maximum in March corresponding with the period of spawning. Not only is tissue hydration greater in larger fish than in smaller, but in addition it lasts longer (about 3 months). The smallest fish are affected for just one month, recovering in April, and it is likely that this effect is due solely to starvation, since the fish are immature. The findings are in accordance with previous observations[3], which showed that the reduction in protein nitrogen was greater in larger fish than in small, owing to the additional need of larger fish to build up gonads on a food supply inadequate even for ordinary metabolism.

There is little doubt that the height of the maximum in Fig. 1, and also its location, depends on the place where the fish were caught, and probably on the year of catching as well. The highest water content that we ever found under natural conditions (87·2 per cent) was in a cod 42 in. long caught off the west coast of Scotland at the beginning of June 1959. The maximum in Fig. 1 is in March 1959, so this isolated observation provides an example of the effect of a different fishing ground.

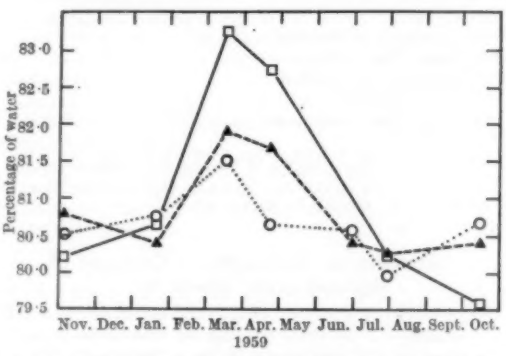

Fig. 1. The water content of cod muscle tissue at different times of the year. ○ · · · ○, Small fish; ▲ – – – ▲, medium fish; □ —— □, large fish. Large fish were not obtainable in June

In a recent review of fish composition[5] it was recommended that in order to obtain the complete picture of the variations in a given constituent, analyses should be carried out at monthly intervals for a year on fish of at least two age groups, the fishing ground being the same throughout. From the data in Fig. 1, the recommendation would not appear to have been unreasonable.

The work described in this communication is part of the programme of the Department of Scientific and Industrial Research.

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[1] Lovern, J. A., and Wood, H., *J. Mar. Biol. Assoc. U.K.*, **22**, 281 (1937).
[2] Hughes, R. B., *J. Sci. Food Agric.*, **10**, 431 (1959).
[3] Ironside, J. I. M., and Love, R. M., *J. Sci. Food Agric.*, **9**, 597 (1958).
[4] Love, R. M., *J. Sci. Food Agric.*, **9**, 617 (1958).
[5] Love, R. M., "The Physiology of Fishes", ed. by Brown, M. E., **1**, 401 (Academic Press, New York, 1957).

Turnover of the Copper and Protein Moieties of Ceruloplasmin

Approximately 95 per cent of the plasma copper in both mouse and man is normally present as a moiety of ceruloplasmin[1]. Ceruloplasmin, an α-globulin containing 0·34 per cent copper, exhibits oxidase activity *in vitro* with a variety of substrates[2] and reversibly releases at least half its copper upon reduction[3]. Whether ceruloplasmin plays a part in the transport of copper or acts as an oxidase *in vivo*, however, is not known. In the work reported here, the function of ceruloplasmin in mice was investigated by determining the rate of exchange of its copper moiety.

Human ceruloplasmin isolated from pooled plasma[3] was kindly supplied by Dr. I. H. Scheinberg. The protein moiety of an aliquot of the preparation was labelled with iodine-131 at *p*H 6·8 using the nitrite method described elsewhere[4]. Iodination did not alter the reactivity of the ceruloplasmin with specific rabbit antisera[5] or change its visible spectrum. More than 98 per cent of the radioactivity of the preparation was precipitable in 10 per cent trichloracetic acid. The iodoceruloplasmin was finally screened in Swiss albino mice for 24 hr. to remove any denatured labelled protein, as suggested by McFarlane[6].

Additional aliquots of human ceruloplasmin were labelled with copper-64 using an exchange method[3]. Of the total radioactivity in these preparations, 92–95 per cent was precipitable with rabbit antiserum.

Labelled mouse ceruloplasmin was obtained by injecting mice intraperitoneally with 100 μgm. copper containing 300 μc. copper-64. Serum collected 24 hr. later had a final activity of 1·7 μc. per ml., of which almost 98 per cent was associated with ceruloplasmin electrophoretically. The pooled serum was used as the preparation of mouse ceruloplasmin in these experiments.

Groups of Swiss albino mice 6–7 weeks old were given 0·5 μc. of one of the labelled ceruloplasmins intravenously; this was equivalent to 20 μgm. human ceruloplasmin labelled with iodine-131, 200 μgm. human ceruloplasmin labelled with copper-64, or 40 μgm. mouse ceruloplasmin labelled with copper-64. The mice were assayed in a well-type sodium iodide crystal scintillator. Three or four mice from each group were killed at intervals; serum and various organs were assayed for radioactivity.

The half-life of iodine-labelled human ceruloplasmin, $t_i\frac{1}{2}$, as determined by its disappearance from the total body or from serum (Fig. 1), was 0·92 day. The copper-64 in human and mouse ceruloplasmins disappeared from serum with half-lives, $t_h\frac{1}{2}$ and $t_m\frac{1}{2}$, of 0·75 and 0·85 days, respectively. With human ceruloplasmin at least, copper-64 disappeared more rapidly than the protein moiety was degraded, suggesting a slow exchange of ceruloplasmin copper in the serum; the half-life of this exchange, $t\frac{1}{2}$, was calculated:

$$t\frac{1}{2} = \frac{t_i\frac{1}{2} \cdot t_h\frac{1}{2}}{t_i\frac{1}{2} - t_h\frac{1}{2}} = 4{\cdot}1 \text{ days}$$

From 2 hr. following the injection of labelled human ceruloplasmin to the end of the experiment, less than 1 per cent of the serum radioiodide and less than 2 per cent of the serum radiocopper were not precipitable with rabbit antiserum versus human ceruloplasmin[5].

It can be seen from Fig. 1 that the amount of ceruloplasmin in the total body after distribution

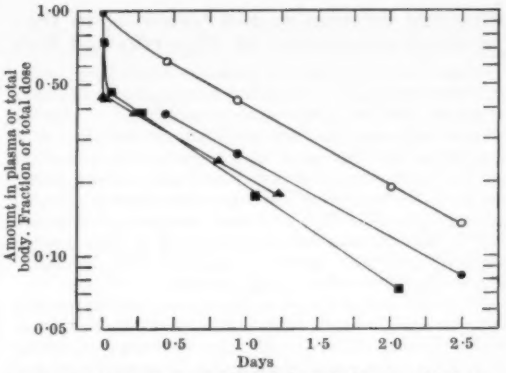

Fig. 1. The disappearance of labelled ceruloplasmins after intravenous injection in mice. From total body: human ceruloplasmin labelled with iodine-131 (○). From plasma: human ceruloplasmin labelled with iodine-131 (●); human ceruloplasmin labelled with copper-64 (■); mouse ceruloplasmin labelled with copper-64 (▲)

of the protein in the body fluids was approximately twice the amount present in the serum. Since the average serum ceruloplasmin concentration in these mice was 13 mgm. per 100 ml. serum and the plasma volume was 1·14 ml., the total ceruloplasmin pool was 0·3 mgm. containing 1·0 μgm. copper. With a half-life of 4·1 days, the amount of copper exchanged within the ceruloplasmin pool per day was 0·17 μgm. The mice studied here ingested 45–75 μgm. copper a day in the diet they received; of this, tracer studies with copper-64 indicated that they absorbed a minimum of 4·5–7·5 μgm. copper a day[7]. It is clear that the amount of ceruloplasmin copper exchanged per day was less than 4 per cent of the amount of copper absorbed from the intestinal tract. This would appear to militate against the suggestion that absorption of copper from the intestines is inhibited or controlled by extensive dissociation of ceruloplasmin copper in the intestinal plasma[3]. The small exchange of ceruloplasmin copper would also indicate that ceruloplasmin does not function to transport copper from the intestines unless the intestines synthesize ceruloplasmin. But even the synthesis of ceruloplasmin by one tissue with subsequent release of its copper in other tissues through degradation could result in the transport of only 1·5 μgm. copper per day.

On the other hand, the observed exchange in ceruloplasmin copper, although quite small, suggests that ceruloplasmin may act, at least in part, as an oxidase *in vivo*.

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[1] Holmberg, C. G., and Laurell, C.-B., *Acta Chem. Scand.*, **2**, 550 (1948).
[2] Holmberg, C. G., and Laurell, C.-B., *Acta Chem. Scand.*, **5**, 476 (1951).
[3] Scheinberg, I. H., and Morell, A. G., *J. Clin. Invest.*, **36**, 1193 (1957).
[4] Gitlin, D., Janeway, C. A., and Farr, L. E., *J. Clin. Invest.*, **35**, 44 (1956).
[5] Scheinberg, I. H., and Gitlin, D., *Science*, **116**, 484 (1952).
[6] McFarlane, A. S., *Biochem. J.*, **62**, 135 (1956).
[7] Gitlin, D., and Hughes, W. L. (unpublished results).

Effect of Adrenaline and Insulin upon the Oxygen Consumption of Hyperthyroid Rats

It has long been known that the administration of thyroxine to rats, or any other species, is followed by an increase in their basal metabolic rate. In the present experiments rats fed thyroid were found to respond to the injection of adrenaline by a further marked and sustained rise in oxygen consumption. No such increase could be demonstrated in normal control animals. This increased sensitivity of hyperthyroid rats to adrenaline, as reflected in their oxygen consumption, was used to examine the release of adrenaline in insulin hypoglycæmia.

Male and female black-hooded rats (average weight 167 gm.) were used. The addition of thyroxine to their drinking water (0·125 mgm./100 ml.) for seven days before the experiment resulted in a 40 per cent increase in oxygen consumption (from 25 to 35 ml./5 min.). The rats were given no food and only limited water for 18 hr. prior to the experiment. The apparatus used was a modification of the spirometer described by Holtkamp *et al.*[1]; this permitted the oxygen consumption of individual rats to be recorded separately. The rats were allowed to become accustomed to the apparatus for 40–60 min. and thereafter their resting oxygen consumption was determined over a period of 1 hr. The animals were then removed, injected and immediately returned to the spirometer. Following this disturbance there was a transient rise in oxygen consumption; but control experiments in which saline was injected (4 rats, Fig. 1) showed that this was insignificant when compared with the increases following the injection of adrenaline or insulin.

The subcutaneous injection of 50 μgm. of DL-adrenaline into four rats fed thyroid was followed by a considerable rise in the consumption of oxygen (Fig. 1). In four normal rats, the change in oxygen consumption after the injection of the same quantity of adrenaline was insignificant.

The injection of insulin (0·125 unit) subcutaneously into six hyperthyroid rats caused a conspicuous increase in the consumption of oxygen (Fig. 2). No such increase could be demonstrated in four normal animals given the same dose of insulin. This rise in oxygen consumption produced by insulin in rats fed thyroid was prevented by the simultaneous intraperitoneal injection of 1·5 ml. of a 30 per cent solution of glucose into a series of eight similar animals (Fig. 2). Any animal that developed hypoglycæmic convulsions was excluded from the series of experiments.

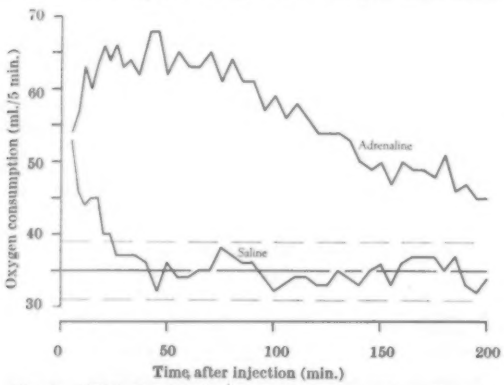

Fig. 1. Effect of adrenaline upon the oxygen consumption of hyperthyroid rats. Upper curve, effect of 50 μgm. of DL-adrenaline in 0·1 ml. saline. Mean of four experiments. Lower curve, effect of the injection of 0·1 ml. saline. Mean of four experiments. The broken lines are drawn two standard deviations from the mean of the resting oxygen consumption

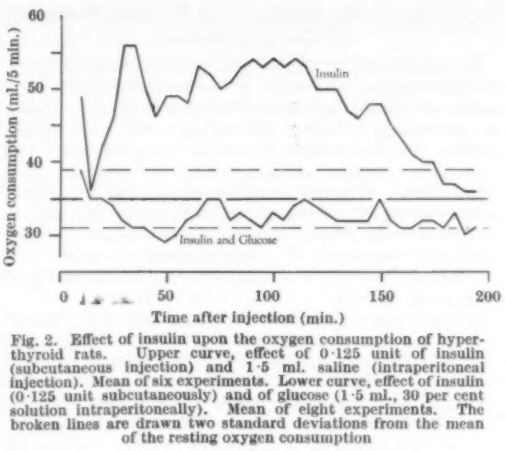

Fig. 2. Effect of insulin upon the oxygen consumption of hyperthyroid rats. Upper curve, effect of 0·125 unit of insulin (subcutaneous injection) and 1·5 ml. saline (intraperitoneal injection). Mean of six experiments. Lower curve, effect of insulin (0·125 unit subcutaneously) and of glucose (1·5 ml., 30 per cent solution intraperitoneally). Mean of eight experiments. The broken lines are drawn two standard deviations from the mean of the resting oxygen consumption

It has been shown[2] that the increased oxygen consumption in the hyperthyroid state could be reduced to normal levels by a total sympathetic block and thereafter restored to the original level by infusion of adrenaline or noradrenaline. As large doses of adrenaline are required to produce even a transient rise in oxygen consumption in normal animals, it was suggested that hyperthyroid animals were unduly sensitive to the calorigenic effects of adrenaline. The sustained and pronounced increase in oxygen consumption following injection of adrenaline into hyperthyroid rats and the insignificant increase produced by the same dose of adrenaline in normal animals, shown in the present experiments, would accord with this suggestion.

The rise in oxygen consumption following the administration of insulin was prevented when the blood sugar was maintained by an injection of glucose; it was therefore inferred that this effect was not a direct consequence of the presence of insulin, but was secondary to the hypoglycæmia produced by this hormone. The demonstration of the release of adrenaline during hypoglycæmia[3] and the evident sensitivity of rats fed thyroid to this agent would imply that the rise in oxygen consumption after the injection of insulin into such animals may be attributed to the secretion of adrenaline. It would appear, therefore, that the oxygen consumption of hyperthyroid rats may be used as an index of changes in the rate of secretion of adrenaline following physiological stimuli.

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[1] Holtkamp, D. E., Ochs, S., Pfeiffer, C. C., and Heming, A. E., *Endocrinol.*, **56**, 93 (1955).

[2] Brewster, W. R., Isaacs, J. P., Osgood, P. F., and King, T. L., *Circulation*, **13**, 1 (1956).

[3] Cannon, W. B., MacIver, M. A., and Bliss, S. W., *Amer. J. Physiol.*, **59**, 46 (1924).

Correlation between the Electrolyte and Water Content of the Organs and Hypertension after Administration of Corticosteroids

It has been reported that, with renal hypertension, retention of sodium and loss of potassium occurred in the musculature[1-4] and that the same changes in electrolyte metabolism appear after application of hypertension-inducing corticosteroids[5-9]. Therefore these alterations in the inorganic metabolism were considered to be the direct cause of hypertension[10,11]. Tobian *et al.*[12] found an increase in sodium, potassium and water content of the aorta in all forms of experimental hypertension and concluded this to be the cause of hypertension.

In connexion with other researches in the rat, we examined three corticosteroids which influence the electrolyte turnover and the blood pressure in different ways. We also studied their effect on sodium, potassium and water content in the aorta in order to detect which of the changes mentioned above in the electrolyte concentration is related to the blood pressure. The corticosteroids used were deoxycorticosterone acetate (5 mgm./100 gm. body-weight on the first day, then 2·5 mgm.), cortisone acetate (the same dosage) and aldosterone acetate (0·025 mgm./100 gm. body-weight). The treatment lasted 10 days. The daily sodium intake was 75–150 mgm. Sodium, potassium and water content was determined in serum, striated muscle, skin, aorta and in the urinary bladder as another organ containing smooth muscle. With the applied dosage of deoxycorticosterone acetate, the blood pressure remains unchanged in the normal rat (Table 1); as is known, the sodium content of striated muscle is increased and the potassium content is decreased. In the aorta, both sodium and water concentration were increased, whereas the potassium concentration was unchanged.

With adrenalectomized animals the same dosage already produces hypertension. In this case, the sodium as well as the potassium and water content of the aorta is increased; in the urinary bladder an increase in sodium only can be established. It should be emphasized that, by means of the double dosage, hypertension occurs even in normal rats without supplementary supply of sodium chloride.

Cortisone acetate causes hypertension in normal as well as in suprarenalectomized animals. The organs show a depletion of sodium and of potassium, the depletion of potassium being less important than after application of deoxycorticosterone acetate.

It is striking that although the animals survive, the loss of sodium is even more marked than with non-treated adrenalectomized animals. In the aortic wall also the sodium decreases; the potassium, on the contrary, increases strongly.

Aldosterone acetate provokes a retention of sodium in all the organs examined, including the aorta, where the potassium content is almost normal; however, blood-pressure remains unchanged. Thus the experiments show that the variations in sodium concentration of the organs in general or especially in the aortic wall are not conclusive for the production of high pressure.

Table 1

	Blood pressure		Striated muscle			Aorta*			Bladder*		
	Before treatment	After treatment	Na m.Eq.	K m.Eq.	H_2O (per cent)	Na m.Eq.	K m.Eq.	H_2O (per cent)	Na m.Eq.	K m.Eq.	H_2O (per cent)
Normal (10)	128 ± 3·2		23·45 ± 0·2	105·1 ± 0·85	76·0 ± 0·2	265·2 ± 0·8	149·2 ± 0·55	63·8 ± 0·6	310·9 ± 0·85	352·5 ± 0·7	78·4 ± 0·3
Normal + DOCA (7)	131 ± 3·5	135 ± 3·8	30·05 ± 0·75	88·0 ± 0·95	76·0 ± 0·25	299·3 ± 0·55	147·7 ± 0·65	66·5 ± 0·5	304·3 ± 0·9	354·5 ± 0·6	79·3 ± 0·2
Adrenalectomized + DOCA (7)	127 ± 3·2	168 ± 2·5	32·86 ± 0·5	87·4 ± 1·2	76·33 ± 0·3	313·3 ± 0·85	171·6 ± 0·75	67·7 ± 0·2	334·0 ± 1·2	358·0 ± 0·8	78·6 ± 0·3
Normal + CA (7)	130 ± 2·8	195 ± 4·2	17·90 ± 0·35	97·8 ± 0·5	75·3 ± 0·5	240·1 ± 0·8	171·5 ± 0·4	63·22 ± 0·2	254·0 ± 0·7	352·1 ± 0·6	78·7 ± 0·2
Adrenalectomized + CA (7)	127 ± 3·2	205 ± 4·0	17·1 ± 0·45	101·7 ± 0·6	75·1 ± 0·45	236·2 ± 0·95	180·5 ± 0·55	62·2 ± 0·25	225·2 ± 0·8	360·5 ± 0·7	77·4 ± 0·2
Normal + AA (5)	132 ± 3·5	130 ± 3·0	25·6 ± 0·4	98·6 ± 0·5	75·29 ± 0·3	294·0 ± 0·9	151·2 ± 0·65	67·8 ± 0·3	345·3 ± 0·9	352·7 ± 1·0	79·2 ± 0·3
Adrenalectomized + AA (5)	128 ± 3·1	122 ± 3·0	30·5 ± 0·4	91·5 ± 0·2	76·1 ± 0·3	318·2 ± 0·85	153·3 ± 0·4	68·5 ± 0·4	351·4 ± 0·95	349·1 ± 0·85	79·7 ± 0·2

* M.equiv./kgm. dry tissue.

DOCA, Deoxycorticosterone acetate; CA, cortisone acetate; AA, aldosterone acetate.

The sodium content in the aorta can be high with normal blood pressure or even low with high blood pressure. On the contrary, the retention of potassium increases parallel with hypertension. It is assumed that the increase of potassium in the aorta wall causes a rise of the tension of the vascular muscle and therefore hypertension. In rats the diameter and length of the large vessels were measured by arteriography. The increase in tension to induce the blood-pressure determined was evaluated.

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[1] Eichelberger, L., *J. Exp. Med.*, **77**, 205 (1943).
[2] Laramore, D. C., and Grollman, A., *Amer. J. Physiol.*, **161**, 278 (1950).
[3] Leonards, J. R., and Heisler, C. R., *Fed. Proc.*, **11**, 247 (1952).
[4] Orbison, J. L., Christian, C. L., and Peters, E., *Arch. Path.*, **54**, 185 (1952).
[5] Selye, H., Hall, C. E., and Rowley, E. M., *Canad. Med. Assoc. J.*, **49**, 88 (1943).
[6] Thorn, G. W., Engel, L. L., and Eisenberg, H., *J. Exp. Med.*, **68**, 161 (1938).
[7] Thorn, G. W., Engel, L. L., and Eisenberg, H., *Bull. Hopkins Hosp.*, **64**, 155 (1939).
[8] Gaunt, R., Birnie, J. H., and Eversole, W. J., *Physiol. Rev.*, **29**, 281 (1949).
[9] Overman, R. R., *Physiol. Rev.*, **31**, 285 (1951).
[10] Braun-Menendez, E., and Covian, M. R., *Rev. Soc. arg. Biol.*, **24**, 31 (1948).
[11] Braun-Menendez, E., Ciba Foundation Symposium on Hypertension. 238 (Churchill, London, 1954).
[12] Tobian, L., and Binion, J. T., *J. Clin. Invest.*, **33**, 1407 (1954). Tobian, L., and Redleaf, P. D., *Amer. J. Physiol.*, **192**, 325 (1958); **189**, 451 (1957). Tobian, L., and Binion, J. T., *Circulation*, **5**, 754 (1952).

Effect of Potassium on Muscle Tension, especially on that of Vascular Muscle

In the previous communication it was shown that in corticosteroid-induced hypertension in the rat, of all the electrolyte shifts, only potassium increase in the aorta wall was characteristic. In striated muscle or in other organs containing smooth muscle, the potassium content either decreases or remains unchanged.

It has been assumed that the increase of concentration of potassium in the vascular muscle causes a tension increase and, as a result, high pressure. Because of this the following experiments were done *in vitro*: (*a*) the effect of potassium on muscle tension; (*b*) qualitative differences concerning the effect of potassium on different muscle types were investigated.

The experiments were especially carried out on carotis strips of cattle, on the musculus rect. abd. of the frog and the rat as well as on the muscle of large intestine of the rat in Tyrode bath, at adequate temperature and with known, variable initial tension. The changes in tension were registered with a transducer fitted perpendicularly to the surface of the examined tissue.

If the potassium content of the Tyrode solution is increased, the tension of the vessel strip increases too. Contrary to Raab's findings[1] the increase of tension can last up to 90 min. according to potassium content and will last as long as potassium is not changed (Fig. 1).

The latent period, the steepness of the tension increase and the total tension depend on the potassium of the bath solution. The maximal tension is attained with a concentration of 60–70 m*M* (Fig. 2). An equilibrium between the bath solution and the extracellular space is established after about 5 min. The increase in tension initiated by potassium and the decrease after removal of it are not influenced by the oxygen content of the bath solution, as is the case with tension caused by electric stimulation or epinephrine. The influence of potassium on striated muscle or on smooth muscle is different. With skeletal muscle as well as with smooth muscle of large intestine only a temporary increase in tension could be produced with the concentrations used. In the muscle of large intestine 30 m*M* potassium first produces an increase in tonus, then a rhythmic fluctuation in tonus.

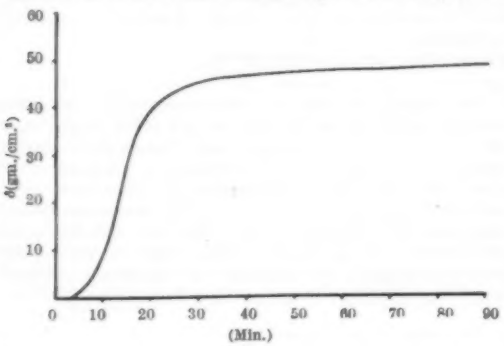

Fig. 1

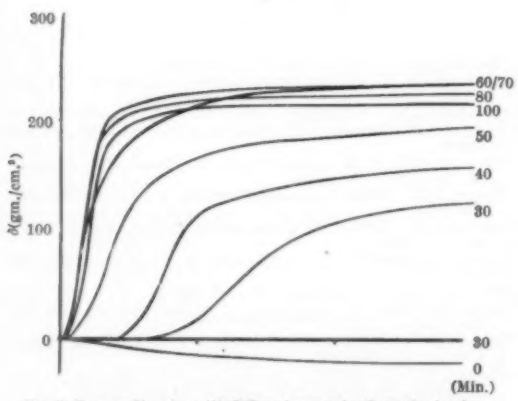

Fig. 2. Course of tension with different concentrations of potassium. Figures on the right indicate m*M* [K]

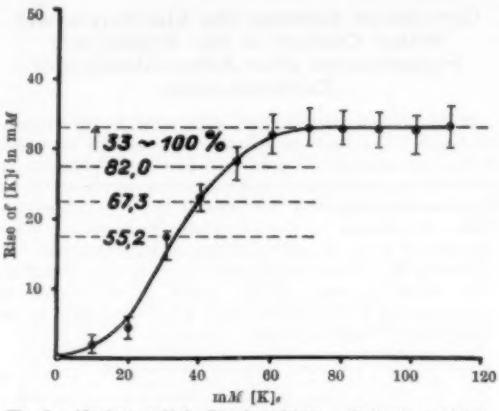

Fig. 3. Abscissa, m*M* in Tyrode solution. Ordinate, m*M* $[K]_i$ increase, after 30 min.

Since on one hand with high-pressure animals the intracellular potassium in the aortic wall is increased, and on the other, as shown, potassium has different effects according to muscle type, the vascular muscle and the muscle of large intestine were examined in regard to their permeability to potassium. The $[K]_i$ was calculated from the total content of $K - [K]_e$. The value of the extracellular space was determined with thiosulphate. As shown in Fig. 3, potassium already penetrates into the vascular muscle when the [K] of the bath solution amounts to 10 m*M* and saturation occurs with a [K] of 60–70 m*M*. If we set the maximum value of the tension increase (Fig. 2) and the $[K]_i$ (Fig. 3) equal to 100, it will be found that the relative tension is proportional to the relative $[K]_i$ (Fig. 3). The course of penetration of potassium into the muscle fibre and of that of tension are similar within a given concentration of the bath solution. No potassium enters the muscle of large intestine of the rat under the same experimental conditions.

It is assumed that two different functional systems are present in the vascular muscle, the contractile system for the transient regulatory tension and the tonic system for the continuous tension. The tension of the contractile system seems to depend on the concentration of potassium in the muscle.

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[1] Raab, E., *Pflügers Arch.*, **215**, 651 (1927).

HÆMATOLOGY

Sedimentation of Blood without Anticoagulants

SEDIMENTATION of blood usually occurs only after anticoagulants have been added to prevent clotting and allow time for settling. In devising a technique to study thrombosis *in vitro*[1], an apparatus was made for collecting blood free of tissue thromboplastin and without the addition of anticoagulants. It was incidentally observed that coagulation of blood so collected was delayed sufficiently long for sedimentation to occur. A similar apparatus for collecting blood to study coagulation has been reported by Spaet, Cintron and Kropatkin[2].

Venous blood is drawn directly into a polyvinyl plastic tube through an attached 19-gauge thin-wall, silicone-coated needle. On the end of the tube (opposite the needle) a silicone-coated, three-way stopcock is attached and an additional length of plastic tubing is attached to the stopcock (Fig. 1). As blood flows through the tube, air is pushed out by the advancing column of blood. 2 ml. of blood are allowed to flow out of the tube and are discarded; the end of the tube is clamped and the needle withdrawn from the vein.

No mixing of blood takes place during collection and none of the blood in the tube is exposed to air. By attaching an empty syringe to the stopcock, blood contained in the long section of tubing may be pumped into other containers for further study (Fig. 1). The short section of tubing is clamped off at each end, removed from the stopcock, and hung in a vertical position. The blood in this clamped-off tube will not clot for 1½–2 hr. in most cases and even longer in some instances. Since the blood coagulates so slowly it will settle out rapidly into two distinct layers of plasma and cellular elements (Fig. 2).

The tubing used is a clear polyvinyl chloride plastic tube that measures 0·133 in. in nominal internal diameter (the tubing was kindly supplied by Mead Johnson and Co., Evansville, Ind.). The tubing varies slightly in internal diameter from section to section by volume measurement. 1 ml. fills 10·5–11·4 cm. of tubing. The long section of tubing

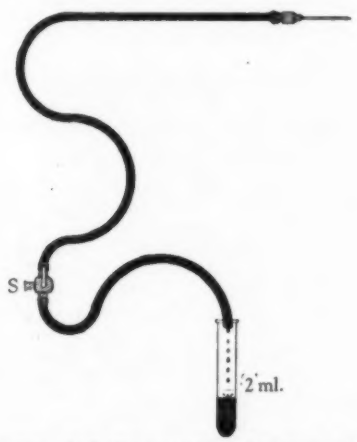

Fig. 1. Diagram of collection apparatus. (*S*), Three-way stopcock. No suction is required for withdrawing blood. Flow can be controlled by pinching the end of the tube

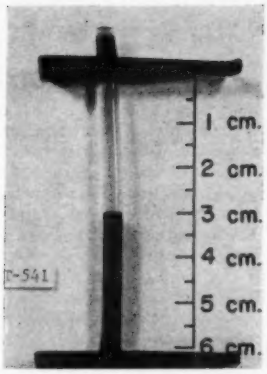

Fig. 2. Sedimentation tube 30 min. after collection of blood from a 52-year-old Negro male with arrested pulmonary tuberculosis. Sedimentation of 3·0 cm. or 50 per cent of the column has occurred. The blood in the upper section beyond the clamp clotted at 45 min. The blood in the enclosed section was not clotted at 2 hr.

used is 64 cm. in length and the short section, 28 cm. A total of 10–11 ml. of blood (including discarded blood) usually is withdrawn. The short section of tubing is marked off at 6-cm. intervals, and clamps are attached to separate the blood column into four equal compartments. Sedimentation occurs in all four compartments and the column of clear plasma in the upper compartment is measured at 30 min. Although there usually is some increase in fall after 30 min., most of the sedimentation has occurred by this time and readable values are obtained. The lower three compartments are opened at 1-hr., 1½-hr. and 2-hr. intervals, respectively, to check for the presence of clotting.

Sedimentation-rates have been determined on the blood of 40 male and 71 female patients of the Out-Patient Clinic of the Eugene Talmadge Memorial Hospital of the Medical College of Georgia. Most of the patients were pre-operative and post-operative and a few were prepartum and postpartum cases. The amount of sedimentation in 30 min. averaged 1·8 cm. or 30 per cent of the 6-cm. column in male patients and 1·9 cm. or 31·6 per cent of the column in female patients. In all cases the blood did not clot for 1 hr. or longer. In one instance no sedimentation occurred in 30 min. even though the blood did not clot for 2 hr. The range of sedimentation in 30 min. varied from 0·5 cm. to 4·0 cm. All experiments were done at room temperature, 21–27° C.

Why is coagulation delayed long enough for sedimentation to occur using this method? Several factors are probably important: (1) Silicone-treated metal and plastic tubing are used. Both silicone and plastic provide non-wettable surfaces that retard clotting. (2) Contamination of the blood with tissue thromboplastin is avoided by allowing the first 2 ml. of blood to pass out of the tube during collection. (3) The blood does not make contact with air. According to an early observation of Babington[3], blood collected under olive oil would "enable its red particles to settle more quickly than would otherwise be the case". Müller[4] interpreted this observation to mean that the delayed coagulation was due to exclusion of air. In the method here reported, all air is excluded from the sedimentation tube because air is pushed out of the tube during collection of the blood. The blood in the upper end of the tube beyond the clamp is exposed to air. This blood usually

coagulates in 15–45 min. and always coagulates sooner than the blood in the enclosed part of the tube (Fig. 2).

Settling of relatively stagnant blood in blood vessels may be an important factor in the pathogenesis of venous thrombosis, especially in dependent veins of immobilized patients. Fahraeus[5] demonstrated rapid sedimentation of blood in a vein by applying two separated tourniquets on the forearm that was held in a vertical position. In pregnant women or diseased individuals, clear plasma could be obtained in 15 min. by venipuncture just below the uppermost tourniquet. Convincing evidence that settling of blood occurs in horizontal blood vessels in conditions such as stasis has been reported by Harding and Knisely[6]. It is interesting that sedimentation of blood in the apparatus described here was first observed when the tube was horizontal. Settling to the lower side of the tube occurred in a few minutes. Sedimentation of blood as reported here provides a method for studying the suspension stability of stagnant blood *in vitro* without anticoagulants.

The suspension stability of blood is known to be affected by the type and concentration of anticoagulant used in the various sedimentation methods that require anticoagulants[7]. The sedimentation-rate of incoagulable hæmophilic blood can be altered by the addition of heparin or sodium citrate[8]. It is important to know whether or not sedimentation methods using anticoagulants give values paralleling those with the method here described. This method is easily applicable to such a study of both normal and abnormal blood. We are at present using this method to record sedimentation on all specimens of blood used in our *in vitro* thrombosis studies[1], and these results will be reported.

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[1] Chandler, A. B., *Lab. Invest.*, **7**, 110 (1958).
[2] Spaet, T. H., Cintron, J., and Kropatkin, M., *J. Lab. and Clin. Med.*, **54**, 467 (1959).
[3] Babington, B. G., *Med. Chir. Trans.*, **16**, 293 (1830).
[4] Müller, J., "Elements of Physiology", 143 (Lea and Blanchard, Philadelphia, 1843).
[5] Fahraeus, R., *Physiol. Rev.*, **9**, 241 (1929).
[6] Harding, F., and Knisely, M. H., *Angiology*, **9**, 317 (1958).
[7] Denson, K. W., *J. Clin. Path.*, **12**, 262 (1959).
[8] Ham, T. H., and Curtis, F. C., *Medicine*, **17**, 447 (1938).

A Fast-moving Hæmoglobin in Hydrops Fœtalis

During a study of the hæmoglobins of premature and still-born infants, a fast-moving hæmoglobin was found in a premature baby which clinically showed the symptoms of hydrops fœtalis associated with anæmia, hepato- and spleno-megaly, and which died shortly after birth.

Hæmoglobin analysis of the blood of the fœtus by the paper electrophoretic method as described previously[1] at *p*H 8·6 showed two hæmoglobin spots. One had the mobility of fœtal hæmoglobin, while the other was a fast-moving hæmoglobin, which was much faster than hæmoglobin *A*, slower than hæmoglobin *H* and resembled hæmoglobin *J* and hæmoglobin Fessas and Papaspyrou. At *p*H 6·5, however, *H* and *J* did not separate from hæmoglobin *A*, while the above-mentioned hæmoglobin was clearly separated, and resembled the hæmoglobin of Fessas and Papaspyrou. It was clearly more cathodic than hæmoglobin *H*. It is a pity that we did not have available for comparison the Barth's hæmoglobin, which was described by Ager and Lehmann[2] as being slightly faster than hæmoglobin Fessas and Papaspyrou and faster than hæmoglobin *J* at *p*H 8·6.

The unusual component in the fœtus mentioned above formed the major component, contrary to what has been found up to now in all the cases of newly born infants carrying a fast-moving hæmoglobin component. The amount of alkali-resistant hæmoglobin examined by the method of Singer *et al.* (1951) was found to be 44·3 per cent of the total amount of hæmoglobin.

The symptoms found in the fœtus closely resembled those seen in hydrops fœtalis caused by antagonism between infants and mother's blood.

Also the pathological signs in the grossly enlarged placenta were those seen in hydrops and erythroblastosis fœtalis. There were no indications of antagonism between mother's and infant's blood. However, both parents showed hæmatological abnormalities resembling those found in thalassæmia minor. No pathological hæmoglobin was detected in either parents. The alkali-resistant hæmoglobin was within normal limits.

This is, so far as we know, the first report of a case of hydrops fœtalis associated with the presence of an unusual hæmoglobin.

We wish to thank Prof. Sutomo Tjokronegoro of the Department of Pathology for his co-operation, and Dr. Lie Hong Gie and the nurses of the Obstetric Department of Jang Seng Ie Hospital for their kind help.

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[1] Lie-Injo Luan Eng and Oey Hoey Glok, *Lancet*, i, 20 (1957).
[2] Ager, J. A. M., and Lehmann, H., *Brit. Med. J.*, i, 929 (1958).

PLANT PHYSIOLOGY

Relation of Sugar Content to Frost-Hardiness in Plants

It has long been said that in various plant cells there is an apparent correlation between the sugar content and frost-hardiness. Because of the difficulties in artificially introducing the appreciable amount of sugar into the plant cell, direct evidence has not yet been obtained to show whether the increase in sugar content is the main cause of the frost-hardiness in plant cells or not.

In artificial frost-hardening of the parenchyma cells in cortex of mulberry tree with a constant length of hardening period, the lower the temperature to which material is exposed, the greater the effectiveness of the hardening treatment becomes. At a definite temperature a relatively longer period of the treatment is more effective than shorter ones within

certain limits. In all these experiments, the increase in frost-hardiness of parenchyma cells is closely proportional to that in their sucrose concentration, but the former is not accompanied by any increase of their soluble protein provided that they are artificially chilled[1].

It becomes necessary, however, to distinguish the effect of low temperature itself and that of the increase in sucrose concentration in considering the frost-hardening process in plant cells as a result of artificial chilling. When pieces of twig cut from a mulberry tree in April were gradually desiccated at room temperature (about 20° C.) for 2–3 days, considerable changes[2] took place in the cortical tissues; namely, decrease both in water content (12 per cent per dry weight) and in starch content (32 per cent per dry weight), increase both in sucrose (50 per cent per dry weight or 83 per cent per wet weight) and in the osmotic concentration (0·35→0·53 *M* sodium chloride). When these pieces of twig were frozen at − 10° C. and − 20° C. for 24 hr., the percentage of survival of the parenchyma cells in the twig pieces was 100 and 70, respectively, but that in normal twig (undesiccated) was 25 and 0, respectively. The cells desiccated at room temperature showed greater increase in frost-hardiness as well as in sucrose concentration than the parenchyma cells in the twig pieces which were chilled at 0° C. for 10 days at that time.

Pieces of twig upon which artificial frost-hardening had been effective[1] were kept about 20 days at 15° C. without desiccation (enveloping them with vinyl sheet) in order to use up the starch granules in the parenchyma cells. Afterwards, these pieces of twig were chilled for 10 days at 0° C., but the parenchyma cells in these twigs did not increase in frost-hardiness or in sucrose concentration. Thus, even at a relatively higher temperature (20–25° C.), the increase in sucrose concentration in the parenchyma cells also resulted in rise of their frost-resistance. On the other hand, whenever any chilling in parenchyma cells resulted in no increase in their sucrose concentration, they could not be hardened further by chilling. These results seem to indicate that, at least in the mulberry tree, low temperature itself has no direct effect on the increase in frost resistance; but the increase of sucrose concentration may be a primary factor in frost-hardiness.

Furthermore, some experiments were made to find out whether the increase of sucrose concentration in plant cells directly results in an increase of their frost-hardiness or not. In order to increase the sucrose content in a cell, twigs of gardenia (*Gardenia jasminoides* Ellis) with 2–3 leaves in September and October (the leaves can withstand freezing at − 5° C. for about 16 hr.) were put in the glucose solution (0·7 *M*) at 25° C. for 24 hr. At the same time, the twig of the control was put in water under the same conditions as mentioned above. The osmotic value of the spongy or the palisade mesophyll cells in a leaf of gardenia was 0·7–0·75 *M* (indicated on the basis of sodium chloride). Osmotic concentration, sugar content and degree of frost-resistance in the palisade and spongy parenchyma cells in leaves of the twig treated in glucose solution or in water are shown in Table 1.

After the treatment the actual increase of osmotic concentration in the cells amounted to about 20–25 atm., with concomitant considerable rise in their frost-hardiness. Furthermore, some other sugars besides glucose, namely, xylose, sucrose, maltose and raffinose, were also found to be able to penetrate into the cells. In such a case, these sugars showed some protective action against freezing injury in gardenia leaf cells.

Table 1. Effect of Glucose Uptake on Frost-Hardiness in Leaf Cells

Treatment	Osmotic concentration† in cells after treatment	Changes in osmotic concentration in cells after treatment	Sugar content in leaves* (per wet weight (gm.))	Percentage of survival after freezing for 24 hr.	
				at −10° C.	at −15° C.
Water (control)	0·7 *M*	−0·05 *M* (decrease)	0·018	0	0
Glucose solution (0·7 *M*)	1·20 *M*	+0·50 *M* (increase)	0·036	100	80

* Exclusive of nerve fraction.
† Mol. indicated on the basis of sodium chloride.

It is obvious from these results that the increase of sugar content in a cell results in that of its frost-hardiness.

In addition, polyols (ethylene glycol, glycerol and mannitol) and acetoamide also showed a protective action against freezing in gardenia leaf cells, but inorganic salts did not.

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[1] Sakai, A., *Low Temp. Res.*, Ser. *B*, **15**, 17 (1957).
[2] Sakai, A., *Low Temp. Res.*, Ser. *B*, **17**, 29 (1959).

Damaging Effect of Drying on *Vicia faba* Seeds

In studies of radiation damage in seeds it has been shown that very dry seeds are more radiosensitive than seeds of medium water content[1,2]. This finding initiated a number of detailed investigations into the radiobiology of very dry seeds to obtain further information on the damaging mechanism of the radiation[3–5]. It has generally been assumed in these studies that drying itself has no harmful effect on the seeds and, for the frequently used barley, this certainly seems to be true according to Caldecott[1], Ehrenberg[2] and Wellington (personal communication). In other species, however, the situation may be rather more complicated. Thus in an earlier paper[6] it has been shown that drying of *Vicia faba* seeds over calcium chloride at 20° C. for 14 days reduces germinability to about 60 per cent and slows down root and shoot growth considerably. Further study of these effects is clearly most important for radiation work in seeds. The effects in *Vicia faba* seeds have, therefore, been investigated in rather more detail.

Seeds of an inbred line of *Vicia faba* var. *minor*, 'Throws M.S. Winter Beans' from Hasler and Co., Ltd., Dunmow, Essex, were used for the experiments. The beans were kept in desiccators in a single layer for periods up to 4 weeks at 20 ± 0·5° C. over the following substances: $NaHSO_4.H_2O$ (laboratory reagent, relative air humidity 52·0 per cent), $CaCl_2.6H_2O$ (analytical reagent, relative air humidity 32·3 per cent), $CaCl_2$ (laboratory reagent, relative air humidity 2·5 per cent).

Water content is sometimes deduced from the conditions and time of storage in desiccators[4]. It was, however, considered necessary to measure the water content of each sample because it is dependent

not only upon the time of storage and the drying agent, but also upon the number of seeds and the volume of the desiccator and other factors. The water content was measured after various periods of storage by crushing samples of 10 beans and drying at 105° C. to constant weight. The measured water content of the various samples used in the experiment varied between approximately 12·0 per cent for beans stored in the room to 7·5 per cent after storage for 4 weeks over calcium chloride, the equilibrium water content over this agent being about 4·5 per cent[7].

For germination experiments samples of 30 beans were soaked at 20 ± 0·5° C., after pricking the testas, for an initial 16 hr. in aerated demineralized water, and for a further 3 days in aerated tap-water with constant supply (about 12 l./hr.). The number of beans germinated was then counted, the criterion for germination being taken as visible splitting of the testa over the tip of the radicle.

For growth measurements, 20 germinated beans (where possible) from each soaked sample were suspended by means of a perforated 'Perspex' shelf in aerated circulating tap-water (constant supply of about 12 l./hr.) in a controlled-temperature water-bath run at 20 ± 0·5° C. After growth for 8 days, root and shoot length were measured. The beans were stored in darkness except for the final pricking, and soaked and grown in darkness except for 5 min. daily at less than 20 ft. candles. These short exposures to light should not affect the ultimate length of the shoot[8].

The scatter diagram for germination percentage in relation to water content (Fig. 1) shows that germination is progressively reduced as the seeds are dried. The correlation coefficient calculated from the results is + 0·789, which for normally distributed data would be very highly significant ($P < 0·001$). Fig. 2 shows the effect of drying on root and shoot growth. A sigmoid curve would be expected and free-hand curves have been drawn in to assist the eye.

Schoorel[9] has pointed out that it cannot be taken for granted that seeds in general maintain their quality better at low moisture-levels, but that some species from temperate zones as well as a number of tropical species have a relatively high water optimum water-content for storage. Evidently *Vicia faba*

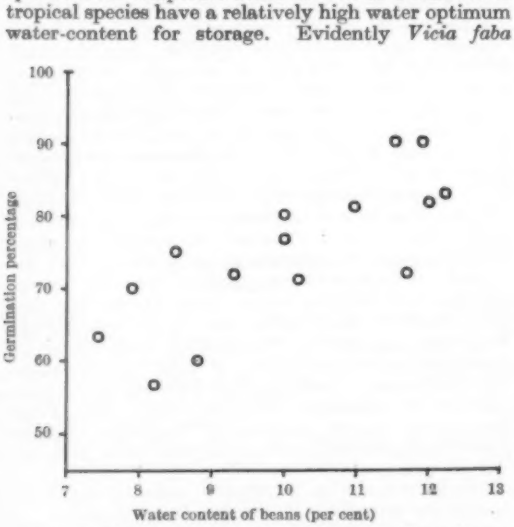

Fig. 1. Germination percentage of *Vicia faba* seeds after soaking for 16 hr. + 3 days, related to water content before soaking

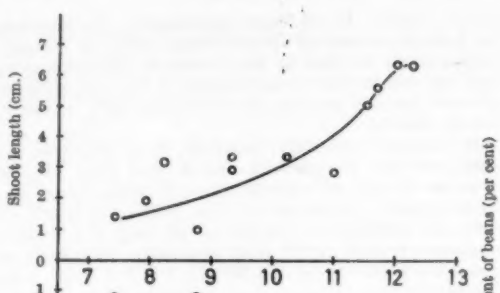

Fig. 2. Root and shoot length of *Vicia faba* after soaking seeds for 16 hr. + 3 days and growing for further 8 days, related to water content before soaking

belongs to the latter category; the seeds cannot be dried much below their normal water content without injury. It may well be that other seeds used in radiobiological experiments have similar properties.

There appears at present to be no explanation of this harmful effect of drying on seeds. It had not escaped us that there might be some direct connexion between this phenomenon and the induction by drying alone of free radicles in the testa[7,10]. This, however, appears to be unlikely, as we have found that removal of the testa before drying does not appreciably reduce the final damage.

The results clearly show that in radiobiological work on dry seeds the effect of drying alone on the seeds may be an important factor to be taken into account in the design and interpretation of experiments.

We wish to thank Dr. P. Wellington of the National Institute of Agricultural Botany, Cambridge, for discussions of the problems involved, and Prof. W. T. Williams of this Department for advice. One of us (W. K.) is also indebted to the Department of Scientific and Industrial Research and the Deutsche Akademische Austauschdienst for a research studentship in Great Britain.

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[1] Caldecott, R. S., *Rad. Res.*, **2**, 339 (1955); **3**, 316 (1955).
[2] Ehrenberg, L., *Botaniska Notiser*, **108**, 184 (1955); *Hereditas*, **41**, 123 (1955).
[3] Caldecott, R. S., Johnson, E. B., North, D. T., and Konzak, C. F., *Proc. U.S. Nat. Acad. Sci.*, **43**, 975 (1957).
[4] Curtis, H. J., Delihas, N., Caldecott, R. S., and Konzak, C. F., *Rad. Res.*, **8**, 526 (1958).
[5] Ehrenberg, A., and Ehrenberg, L., *Arkiv för Fysik*, **14**, 133 (1958).
[6] Klingmüller, W., *Naturforsch.*, **14**, b, 268 (1959).
[7] Klingmüller, W., Lane, G. R., Saxena, M. C., and Ingram, D. J. E., *Nature*, **184**, 464 (1959).
[8] Lane, G. R., and Butler, R. D., *Nature*, **181**, 1080 (1958).
[9] Schoorel, A. F., *Het Landbouwkundig Tijdschrift*, **70**, 117 (1958), and preprint of Twelfth Internat. Seed Testing Convention, Oslo (July 1959).
[10] Klingmüller, W., and Saxena, M. C., *Naturwiss.*, **46**, 475 (1959).

HISTOLOGY

Reciprocal Relationship of Phosphorylase and Oxidative Enzymes in Skeletal Muscle

WHILE investigating various enzyme systems in a series of dystrophic and normal human muscles we observed that individual fibres showed a reciprocal relationship between phosphorylase and oxidative enzyme content. Although other workers have investigated the oxidative enzyme systems of normal[1-3] and dystrophic[4] muscle, no observations have been made on the relationship between the two enzymes.

Biopsies were obtained from the lateral head of the gastrocnemius in fourteen cases of progressive muscular dystrophy of the Duchenne type ('pseudo-hypertrophic' muscular dystrophy) and six normal children. All specimens were immediately quenched in liquid air, stored at − 70° C., and cut within a few hours on a cold microtome at − 20° C. and at 10–15μ.

In an attempt to explain the findings in human muscle a number of different muscles from the rat, pigeon, toad (*Xenopus*) and goldfish were also examined.

The methods employed were those for diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorases[5]; diphosphopyridine nucleotide-linked β-hydroxybutyrate, *iso*citrate, malate, glutamate, α-glycerophosphate, alcohol and lactate dehydrogenases and triphosphopyridine nucleotide-linked *iso*citrate dehydrogenase[6]; succinate dehydrogenase[7]; cytochrome oxidase[8] and glucosan phosphorylase[9].

With the exception of β-hydroxybutyrate dehydrogenase, all the oxidative enzymes and phosphorylase were active in both normal and dystrophic human muscle. Diphosphopyridine nucleotide-diaphorase and lactate dehydrogenase were the strongest.

In dystrophic muscle the majority of abnormally large fibres (100–200μ) reacted weakly for all the oxidative enzymes and strongly for phosphorylase, while the small atrophic fibres (20–30μ) tended to react strongly for oxidative enzymes and weakly for phosphorylase (Fig. 1*A* and *B*). The normal muscle showed a similar variation (Figs. 2 and 3). Longitudinal sections established the uniformity of reaction throughout the length of a fibre.

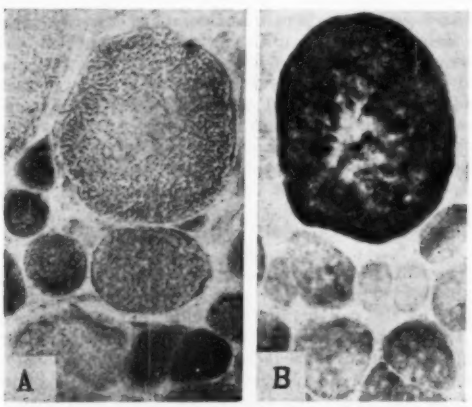

Fig. 1. *A*, Dystrophic muscle. Diphosphopyridine nucleotide-diaphorase (× 234). *B*, Dystrophic muscle. Phosphorylase (× 190)

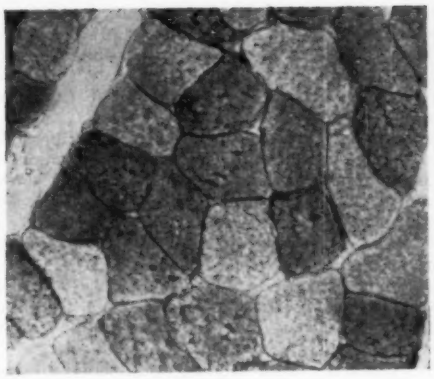

Fig. 2. Normal human tibialis anterior. Lactate dehydrogenase (× 208)

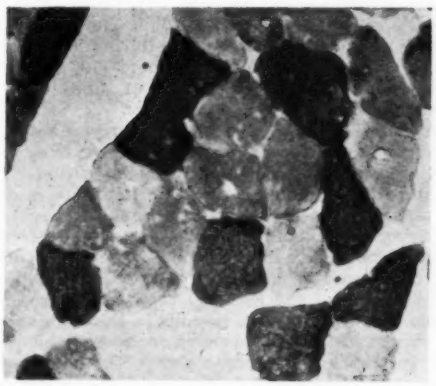

Fig. 3. Normal human tibialis anterior (serial section). Phosphorylase (× 208)

Some normal muscles also contained fibres of intermediate size and enzyme activity, while occasional muscles showed variation in enzyme activity without variation in size (Fig. 2). In a number of dystrophic muscles the reaction for glutamate, malate, α-glycerophosphate, and alcohol dehydrogenases was uniform irrespective of fibre size. This ruled out the possibility of mitochondrial dispersion as a cause of the weaker reaction for the oxidative enzymes in the enlarged fibres.

It appears, therefore, that normal human muscle is composed of two types of fibre; one, generally of smaller diameter, is strong in intramitochondrial oxidative enzymes and weak in phosphorylase, while the other, usually larger, reacts conversely. The atrophic and hypertrophic fibres in muscular dystrophy correspond to these two types respectively. This observation may explain why in muscular dystrophy some fibres hypertrophy while others atrophy. Variations in the proportion of the two types of fibres may account for the earlier and more frequent involvement of certain muscle groups by the dystrophic process.

A reciprocal relationship between phosphorylase and oxidative enzyme activity with the corresponding variation in fibre size was also demonstrated in the muscle fibres of the rat and pigeon. Different muscles contained varying proportions of the fibre types.

not only upon the time of storage and the drying agent, but also upon the number of seeds and the volume of the desiccator and other factors. The water content was measured after various periods of storage by crushing samples of 10 beans and drying at 105° C. to constant weight. The measured water content of the various samples used in the experiment varied between approximately 12·0 per cent for beans stored in the room to 7·5 per cent after storage for 4 weeks over calcium chloride, the equilibrium water content over this agent being about 4·5 per cent[7].

For germination experiments samples of 30 beans were soaked at 20 ± 0·5° C., after pricking the testas, for an initial 16 hr. in aerated demineralized water, and for a further 3 days in aerated tap-water with constant supply (about 12 l./hr.). The number of beans germinated was then counted, the criterion for germination being taken as visible splitting of the testa over the tip of the radicle.

For growth measurements, 20 germinated beans (where possible) from each soaked sample were suspended by means of a perforated 'Perspex' shelf in aerated circulating tap-water (constant supply of about 12 l./hr.) in a controlled-temperature water-bath run at 20 ± 0·5° C. After growth for 8 days, root and shoot length were measured. The beans were stored in darkness except for the final pricking, and soaked and grown in darkness except for 5 min. daily at less than 20 ft. candles. These short exposures to light should not affect the ultimate length of the shoot[8].

The scatter diagram for germination percentage in relation to water content (Fig. 1) shows that germination is progressively reduced as the seeds are dried. The correlation coefficient calculated from the results is + 0·789, which for normally distributed data would be very highly significant ($P < 0·001$). Fig. 2 shows the effect of drying on root and shoot growth. A sigmoid curve would be expected and free-hand curves have been drawn in to assist the eye.

Schoorel[9] has pointed out that it cannot be taken for granted that seeds in general maintain their quality better at low moisture-levels, but that some species from temperate zones as well as a number of tropical species have a relatively high water optimum water-content for storage. Evidently *Vicia faba* belongs to the latter category; the seeds cannot be dried much below their normal water content without injury. It may well be that other seeds used in radiobiological experiments have similar properties.

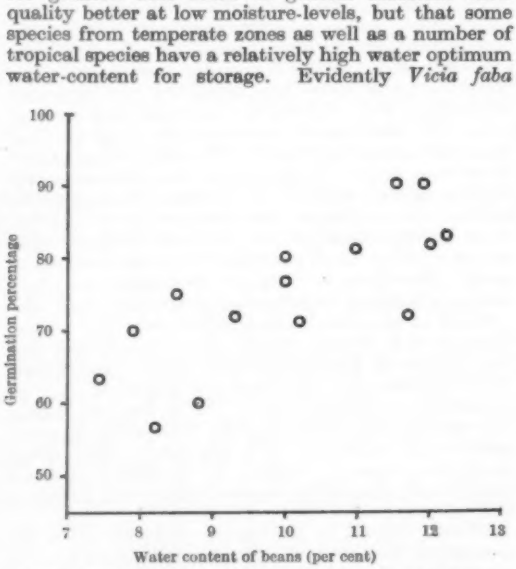

Fig. 1. Germination percentage of *Vicia faba* seeds after soaking for 16 hr. + 3 days, related to water content before soaking

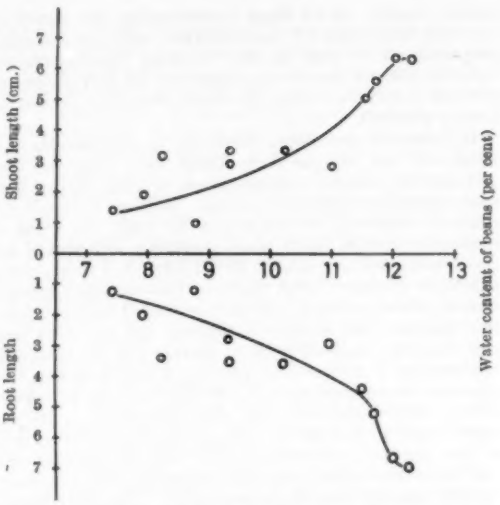

Fig. 2. Root and shoot length of *Vicia faba* after soaking seeds for 16 hr. + 3 days and growing for further 8 days, related to water content before soaking

There appears at present to be no explanation of this harmful effect of drying on seeds. It had not escaped us that there might be some direct connexion between this phenomenon and the induction by drying alone of free radicles in the testa[7,10]. This, however, appears to be unlikely, as we have found that removal of the testa before drying does not appreciably reduce the final damage.

The results clearly show that in radiobiological work on dry seeds the effect of drying alone on the seeds may be an important factor to be taken into account in the design and interpretation of experiments.

We wish to thank Dr. P. Wellington of the National Institute of Agricultural Botany, Cambridge, for discussions of the problems involved, and Prof. W. T. Williams of this Department for advice. One of us (W. K.) is also indebted to the Department of Scientific and Industrial Research and the Deutsche Akademische Austauschdienst for a research studentship in Great Britain.

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[1] Caldecott, R. S., *Rad. Res.*, **2**, 339 (1955); **3**, 316 (1955).
[2] Ehrenberg, L., *Botaniska Notiser*, **108**, 184 (1955); *Hereditas*, **41**, 123 (1955).
[3] Caldecott, R. S., Johnson, E. B., North, D. T., and Konzak, C. F., *Proc. U.S. Nat. Acad. Sci.*, **43**, 975 (1957).
[4] Curtis, H. J., Delihas, N., Caldecott, R. S., and Konzak, C. F., *Rad. Res.*, **8**, 526 (1958).
[5] Ehrenberg, A., and Ehrenberg, L., *Arkiv för Fysik*, **14**, 133 (1958).
[6] Klingmüller, W., *Naturforsch.*, **14**, b, 268 (1959).
[7] Klingmüller, W., Lane, G. R., Saxena, M. C., and Ingram, D. J. E., *Nature*, **184**, 464 (1959).
[8] Lane, G. R., and Butler, R. D., *Nature*, **181**, 1080 (1958).
[9] Schoorel, A. F., *Het Landbouwkundig Tijdschrift*, **70**, 117 (1958), and preprint of Twelfth Internat. Seed Testing Convention, Oslo (July 1959).
[10] Klingmüller, W., and Saxena, M. C., *Naturwiss.*, **46**, 475 (1959).

HISTOLOGY

Reciprocal Relationship of Phosphorylase and Oxidative Enzymes in Skeletal Muscle

WHILE investigating various enzyme systems in a series of dystrophic and normal human muscles we observed that individual fibres showed a reciprocal relationship between phosphorylase and oxidative enzyme content. Although other workers have investigated the oxidative enzyme systems of normal[1-3] and dystrophic[4] muscle, no observations have been made on the relationship between the two enzymes.

Biopsies were obtained from the lateral head of the gastrocnemius in fourteen cases of progressive muscular dystrophy of the Duchenne type ('pseudo-hypertrophic' muscular dystrophy) and six normal children. All specimens were immediately quenched in liquid air, stored at − 70° C., and cut within a few hours on a cold microtome at − 20° C. and at 10–15μ.

In an attempt to explain the findings in human muscle a number of different muscles from the rat, pigeon, toad (*Xenopus*) and goldfish were also examined.

The methods employed were those for diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorases[5]; diphosphopyridine nucleotide-linked β-hydroxybutyrate, *iso*citrate, malate, glutamate, α-glycerophosphate, alcohol and lactate dehydrogenases and triphosphopyridine nucleotide-linked *iso*citrate dehydrogenase[6]; succinate dehydrogenase[7]; cytochrome oxidase[8] and glucosan phosphorylase[9].

With the exception of β-hydroxybutyrate dehydrogenase, all the oxidative enzymes and phosphorylase were active in both normal and dystrophic human muscle. Diphosphopyridine nucleotide-diaphorase and lactate dehydrogenase were the strongest.

In dystrophic muscle the majority of abnormally large fibres (100–200μ) reacted weakly for all the oxidative enzymes and strongly for phosphorylase, while the small atrophic fibres (20–30μ) tended to react strongly for oxidative enzymes and weakly for phosphorylase (Fig. 1*A* and *B*). The normal muscle showed a similar variation (Figs. 2 and 3). Longitudinal sections established the uniformity of reaction throughout the length of a fibre.

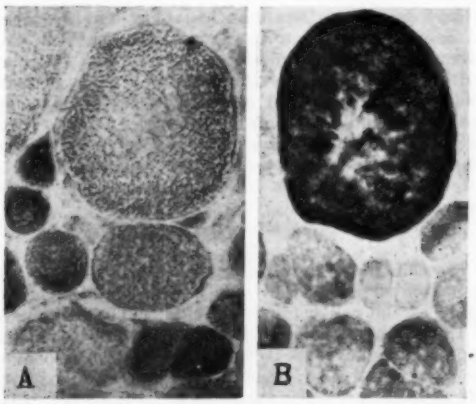

Fig. 1. *A*, Dystrophic muscle. Diphosphopyridine nucleotide-diaphorase (× 234). *B*, Dystrophic muscle. Phosphorylase (× 190)

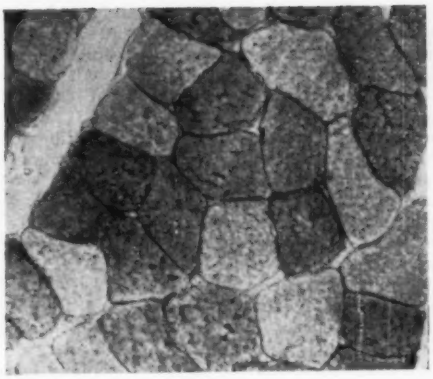

Fig. 2. Normal human tibialis anterior. Lactate dehydrogenase (× 208)

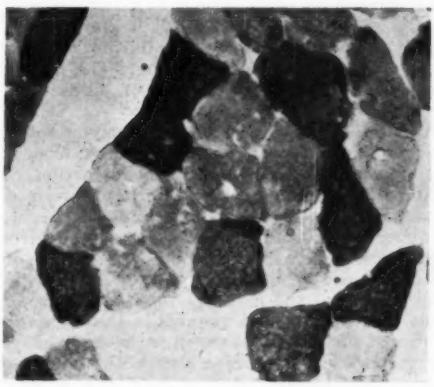

Fig. 3. Normal human tibialis anterior (serial section). Phosphorylase (× 208)

Some normal muscles also contained fibres of intermediate size and enzyme activity, while occasional muscles showed variation in enzyme activity without variation in size (Fig. 2). In a number of dystrophic muscles the reaction for glutamate, malate, α-glycerophosphate, and alcohol dehydrogenases was uniform irrespective of fibre size. This ruled out the possibility of mitochondrial dispersion as a cause of the weaker reaction for the oxidative enzymes in the enlarged fibres.

It appears, therefore, that normal human muscle is composed of two types of fibre; one, generally of smaller diameter, is strong in intramitochondrial oxidative enzymes and weak in phosphorylase, while the other, usually larger, reacts conversely. The atrophic and hypertrophic fibres in muscular dystrophy correspond to these two types respectively. This observation may explain why in muscular dystrophy some fibres hypertrophy while others atrophy. Variations in the proportion of the two types of fibres may account for the earlier and more frequent involvement of certain muscle groups by the dystrophic process.

A reciprocal relationship between phosphorylase and oxidative enzyme activity with the corresponding variation in fibre size was also demonstrated in the muscle fibres of the rat and pigeon. Different muscles contained varying proportions of the fibre types.

The pectoralis muscle of the pigeon, a red muscle, consisted mainly of small fibres (20–35μ) rich in oxidative enzymes and poor in phosphorylase. It contained a few large fibres (40–50μ), with converse activity, located at the periphery of the muscle bundles.

In the toad and goldfish the small fibres were strong in oxidative enzymes as well as phosphorylase, while the large fibres were weak in both enzyme systems. All the muscles of the toad and the white muscles of the fish contained small, intermediate and large fibres. The red muscle of the fish is composed entirely of strongly reactive small fibres.

Assuming that phosphorylase reflects the ability to utilize and synthesize glycogen, it seems that in the larger muscle fibres in warm-blooded animals intrinsic glycogen is the main source of energy. The smaller fibres preferentially use the various substrates of the Krebs cycle. The high lactate dehydrogenase activity in muscle suggests that not all lactic acid produced in muscle is transported to the liver for further oxidation.

In the case of phosphorylase it is known that the inactive precursor is activated during contraction and by such procedures as freezing. The possibility therefore exists that one type of fibre may be preferentially activated.

On the other hand, the reciprocal enzyme activities may represent different phases in a cycle of activity associated with different states of contraction. This would explain the occurrence of fibres of intermediate size and activity. It seems impossible, however, for the hypertrophic and atrophic fibres in dystrophic muscle to be merely different phases of activity of the same fibre type.

The muscle of the fish and toad differs from the muscle of warm-blooded animals in that some fibres are strong in oxidative enzymes as well as phosphorylase, while others are weak in both. The marked variation in diameter between the fibres of different enzyme activity supports the view that these fibres are of different types, as does the consistency of both reaction and size in the red muscle of the fish.

The small muscle fibres with strong oxidative enzyme and weak phosphorylase activity seem to correspond to the red muscle fibres in animals. There is no apparent correlation of fibre types with the fast and slow fibres of amphibia.

A detailed report of this work will be published elsewhere.

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[1] Wachstein, M., and Meisel, E., *J. Biophys. Biochem. Cytol.*, **1**, 483 (1955).
[2] Nachmias, V. T., and Padykula, H. A., *J. Biophys. Biochem. Cytol.*, **4**, 47 (1958).
[3] Ogata, T., *Acta Med. Okayama*, **12**, 216 (1958).
[4] Beckett, E. B., and Bourne, G. H., *Acta Anat.*, **33**, 289 (1958).
[5] Scarpelli, D. G., Hess, R., and Pearse, A. G. E., *J. Biophys. Biochem. Cytol.*, **4**, 747 (1958).
[6] Hess, R., Scarpelli, D. G., and Pearse, A. G. E., *J. Biophys. Biochem. Cytol.*, **4**, 753 (1958).
[7] Pearse, A. G. E., *J. Histochem. and Cytochem.*, **5**, 515 (1957).
[8] Burstone, M. S., *J. Histochem. and Cytochem.*, **7**, 112 (1959).
[9] Takeuchi, T., *J. Histochem. and Cytochem.*, **4**, 84 (1956).

Mitotic Pattern and Histogenesis of the Central Nervous System

Rounded cells which repeat mitosis adding new cells to the tissue are found at the free margin of developing organs, both in plant and animal. In plants the germinal cells form cambium, while in various animal organs the germinal cells are arranged in an epithelium-like layer at the free margin of the tissues[1].

A typical example is observed in the developing central nervous system or the neural tube (Fig. 1). The germinal layer of the neural tube is called germinal epithelium. The cells in this germinal epithelium are supposed to produce new cells which constitute the mantle layer[2]. Close examination of these cells in the germinal epithelium reveals that: (1) the germinal cells are rounded and always found directly beneath the internal limiting membrane; (2) all the nuclei of the germinal cells show mitotic changes from pro-metaphase to early telophase; (3) their cytoplasm appears clear in a usual stain; (4) the direction of the mitosis is always parallel with the internal limiting membrane.

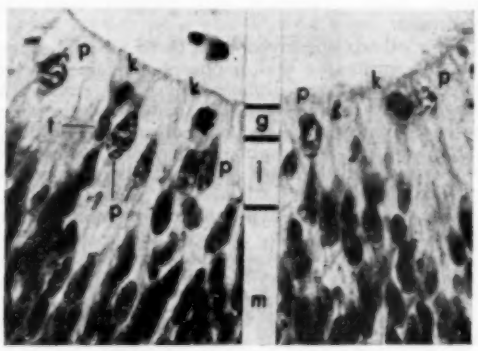

Fig. 1. The wall of the neural tube of the human embryo (C.R., 12 mm.). *g*, Germinal epithelium; *i*, intermediate zone; *m*, mantle layer; *k*, so-called germinal cell showing mitotic nucleus; *p*, prophase nucleus; *t*, nucleus of late telophase

Where an intermediate zone with sparse nuclei exists between the germinal epithelium and the mantle layer (Fig. 1), no resting cell is found in the germinal epithelium; whereas the resting cells are packed densely in the mantle layer. In the intermediate zone between the germinal epithelium and the mantle layer, nuclei at the latter half of telophase and early prophase are to be found. The prophase nucleus is swollen and enlarged, and the telophase nucleus is smaller[3]; but these nuclei in the intermediate zone show smooth transition to the resting cells in the mantle layer on one hand and to the mitotic germinal cells in the germinal epithelium on the other.

These observations can be explained if we assume that the following changes (Fig. 2) occur in sequence as the cells undergo mitosis. The resting cells in the mantle layer rise toward the internal limiting membrane as the cell begins to divide. (The emigration of the germinal cells to the internal limiting membrane during mitosis is considered to be associated with the cytoplasmic changes which round off[4] the mitotic cells, releasing them from the adjacent cells.) The cytoplasm of the cells becomes rounded and takes up less stain than usual. (The rounding off

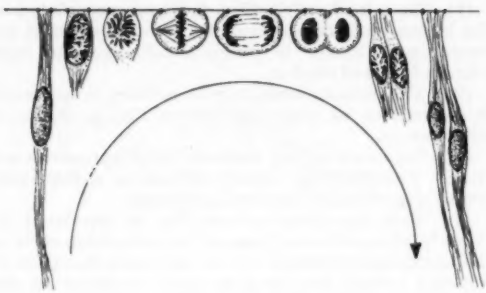

Fig. 2

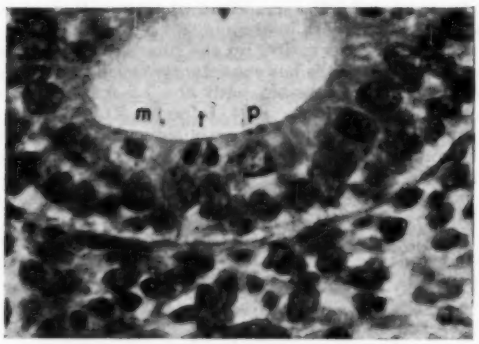

Fig. 3. Mitotic figures in the intestine of the same embryo as Fig. 1. *p*, Prophase nucleus; *m*, metaphase nucleus; *t*, telophase nucleus

of the mitotic cells seems to be related to the solution of the cytoplasm[5,6] and to insufficient supply of adenosine triphosphate to the contractile system in the cytoplasm during mitosis[7].) The chromosomes separate in the direction parallel with the internal limiting membrane, and, when the cells have passed through mitosis, they move back to the mantle layer.

Though this interpretation of cell migration has already been suggested by Sauer[8], his claim and the presence of the same mitotic pattern in a great variety of organs have never attracted attention. I have found this same mitotic pattern (as mentioned in (1)–(4) above) in the following tissues: *Adults*: the epithelium of the efferent duct of the epididymis; the gastric mucosa; the intestinal mucosa; the epithelium of the endometrial gland. *Embryo*: the epithelium of the branchial clefts; the tracheal and lung anlage; the gastric mucosa; the intestinal epithelium (Fig. 3); the epithelium of the bile duct system including the gall-bladder; the epithelium of the pancreas (these six tissues are of endodermal origin); the mesonephric tubule; the mesonephric duct; the metanephric tubule; thickened part of the mesothelium (these four are of mesodermal origin); the epithelium of the nasal pit; Rathke's cleft; the anlage of the retina; the neural tube (these four are of ectodermal origin). The same mitotic pattern is found without distinction of germ layer origin. What is common in these tissues is the fact that they consist of a single layer of cylindrical epithelium. Though the neural tube seems to be many-layered, it can be clearly demonstrated, using Golgi's silver method, that the wall of the tube is actually a modified single-layered epithelium (Fig. 4). Therefore, we conclude that the so-called germinal cells in these epithelial tissues including the neural tube are, in fact, not cells *sui generis* but the actual cells of the epithelium in mitotic phase.

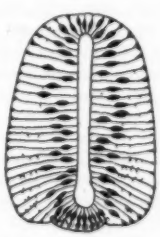

Fig. 4. Cord of chick embryo of the third day, stained by Golgi's method (after Cajal)

Concerning the development of the neural tube, this conclusion introduces a new point of view to the concept of the early histogenesis of the central nervous system, namely, the resting cells in the mantle layer that have been called ependymoblasts and primitive spongioblasts are nothing but the 'germinal cells' in the resting state. This concept, it is believed, contributes to the clearer understanding of the problem of the histogenesis of neurectodermal tumours[9] and of the regeneration of the central nervous system[10].

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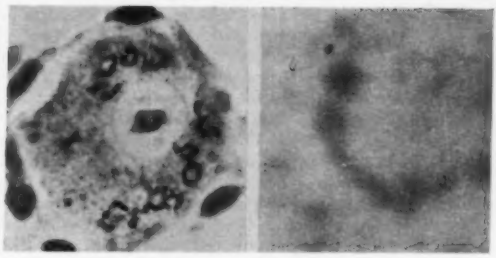
[1] Swann, M. M., *Cancer Res.*, **18**, 1118 (1958).
[2] Ziehen, T., "Hertwig's Hdb. d. vergl. exp. Entw.-gesch. d. Wirbeltieren", **3**, 3 (Jena, 1906).
[3] Brachet, J., "Biochemical Cytology" (1957).
[4] Ris, H., in "Analysis of Development" (Saunders, Phil., 1955).
[5] Marsland, D., in "International Review of Cytology", **5** (Academic Press, New York, 1956).
[6] Heilbrun, L. V., "An Outline of General Physiology" (Saunders, Phil., 1952).
[7] Lettré, H., *Naturwiss.*, **38**, 490 (1951).
[8] Sauer, F. C., *J. Comp. Neurol.*, **62**, 377 (1935).
[9] Fujita, S., *Acta Path., Jap.* (in the press).
[10] Windle, W. F., "Regeneration on the Central Nervous System" (Charles C. Thomas, Springfield, U.S.A., 1955).

BIOLOGY

Electron Microscopy of the Golgi Apparatus

It has become customary in studies of the nerve cell by electron microscopy to apply the term 'Golgi apparatus' to appearances suggesting membranes, granules, or vacuoles[1]. In size, general configuration and osmiophilia the objects so designated cannot readily be recognized as forming part of the large intracellular 'network' familiar to workers with the light microscope. Moreover, adequate controls of these observations are prevented by the short periods (2–12 hr.) of osmication usual in the preparation of tissues for electron microscopy. As is well known, tissues must remain in osmic solutions for days or weeks before the capricious impregnation of the Golgi apparatus results. Further, it has been suggested that the initial buffering of the osmium fixative used in electron microscopy prevents for some reason the classical 'black reaction' of Kopsch (Gatenby, J. B., personal communication, 1958).

After experimentation, this difficulty has been circumvented and it has been found possible to view



(*a*) (*b*)

Fig. 1. *a*, Golgi network in light microscope photograph of a nerve cell (control). Palade fixation with post-osmication 30 days (× 1,200); *b*, portion of the Golgi net (thin section) photographed with the light microscope and further enlarged to × 10,000 for comparison with Fig. 2

with the electron microscope nerve tissues, known from light microscope controls to have undergone the necessary Kopsch reaction. Considerably lengthened schedules of osmication (30 days) followed by soaking in distilled water at 37° C. resulted in adequate impregnation of the apparatus in the controls, while preserving satisfactory fixation of the cytoplasm at the level of resolution of the electron microscope.

Fig. 1*a* is a photomicrograph at (× 1,200) of a dorsal root ganglion cell of a kitten that was fixed in buffered osmium (Palade fluid), and after post-osmication was embedded in wax and cut at 7μ. The cytoplasm is filled with the blackened networks described by Golgi[2]. Fig. 1*b* is from a 2μ section from the same block. In thin sections the network appears as individual thread-like bodies characteristically curved and beaded. One of these bodies photographed with the light microscope is enlarged to × 10,000 for comparison with Fig. 2.

Identically treated pieces of ganglion were embedded in methacrylate and cut to silver/gold sections with the ultramicrotome. On examination with the electron microscope, portions of the Golgi apparatus exactly comparable with those of Fig. 1*b* were easily recognizable during scanning and were selected for micrography (Fig. 2). From an examination of the electron micrographs certain inferences can be drawn.

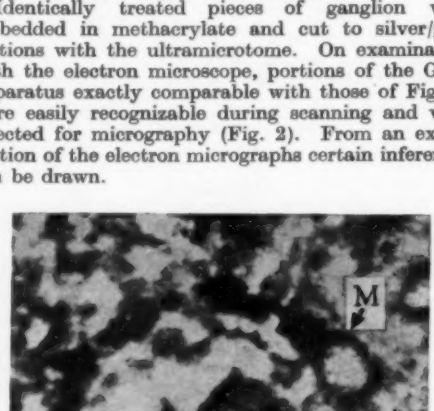


Fig. 2. Electron micrograph (× 22,000) showing Golgi net in close relation to vacuolated mitochondria, *M*

(1) The mitochondria (Fig. 2,*M*) are vacuolated in the kitten and are identical with those recorded by Barton and Causey[3] in ganglion cells fixed for only 2 hr. in buffered osmium.

(2) The mitochondria may be solitary or arranged in aggregates in close apposition like a chain of streptococci.

(3) Segments of the classical Golgi apparatus are clearly recognizable. They appear as a relatively coarse deposition of osmium particles.

(4) This deposition of osmium is unrelated to other 'preformed' structures in the cytoplasm such as the agranular reticulum or the acrosome-like piles of 'parallel plates' described by some workers[1] as the Golgi apparatus in the nerve cell.

(5) A clear space suggesting a shrinkage artefact may accompany the strands of osmium. It does not have a counterpart in the control material.

Repeated observation by the phase microscope on living ganglion cells has consistently failed to visualize any object comparable with the blackened bodies seen in the fixed cells (Figs. 1*a* and *b*, 2). Individual granules or aggregates of granules, comparable with the mitochondria of fixed cells, can however be readily seen. These findings will be discussed and a more extensive publication of the micrographs will appear elsewhere.

I wish to thank Dr. H. D. Purves for assistance and the Medical Research Council (N.Z.) for facilities.

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[1] Gatenby, J. B., and Lufty, R. G., *Nature*, **177**, 1027 (1956).
[2] Golgi, C., *Arch. Ital. Biol.*, **30**, 60 (1898).
[3] Barton, A. A., and Causey, G., *J. Anat.*, **92**, 399 (1958).

Abbreviated Development in a Pinnotherid Crab

Atkins[1] has described the post-embryonic development of two British species of *Pinnotheres*. In *P. pisum* there are four zoœal stages and one megalopa, and in *P. pinnotheres* there are two zoœæ and a megalopa. In *P. pisum* the megalopa stage was reached six weeks after spawning and in *P. pinnotheres* it was reached after an interval of 21–26 days. Atkins quotes Sandoz and Hopkins[2] and Hart[3] to the effect that *P. ostreum* takes 21 days from hatching to megalopa and *P. taylori* 28 days from hatching to young crab.

P. moseri Rathbun is a tropical crab found in the atrial cavity of a large simple ascidian, *Ascidia nigra*. Recent observations show that the post-embryonic development of this crab is unusually rapid. Two berried females brought into the laboratory on October 21, 1959, liberated zoœæ during the nights of October 28 and 30, respectively. In both cases the zoœæ correspond closely to the fourth zoœal stage of *P. pisum* as described by Atkins, with the exception that the rostral spine is short, and lateral spines are absent. There are four swimming setæ on the maxillipeds and the thoracic limbs are well developed, the first pereiopod being chelate: in living specimens the thoracic limbs tend to be hidden under the carapace (Fig. 1).

The first batch of zoœæ moulted to megalopas in the early morning of October 30, giving a maximum

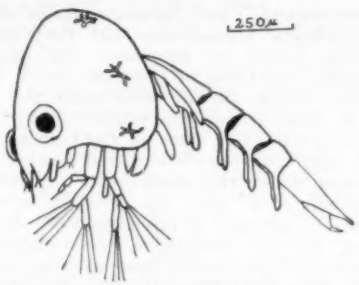

Fig. 1. *Pinnotheres moseri*, newly hatched zoœa drawn from a living specimen with the abdomen extended. Four of the thoracic appendages are visible and also portions of the right maxillipeds appearing below those of the left side

time between hatching and attainment of megalopa of 36 hr. In the second batch of larvæ moulting to the megalopa was first seen at 17.30 hr. on October 31, thus giving a maximum of about 24 hr. from hatching to megalopa. As both batches hatched during the night the exact times are not known. The larvæ in both cases were kept at about 29° C., which was within 1° C. of sea water temperatures in October.

The post-embryonic development of *P. moseri* is thus considerably abbreviated when compared with what is known of other species of Pinnotheres. The significance of such abbreviation cannot at present be assessed; but it is pertinent to point out that *Ascidia nigra* is confined in its distribution to sheltered water such as enclosed bays and lagoons and is absent from communities of animals in more open areas. If *P. moseri* is an obligate commensal in *A. nigra* and cannot live in other hosts, an abbreviated larval development might be advantageous in preventing the larvæ from dispersing too far away from the concentrations of its host population.

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[1] Atkins, D., *Proc. Zoo. Soc. Lond.*, **124** (4), 687 (1955).
[2] Sandoz, M., and Hopkins, S. H., *Biol. Bull., Woods Hole*, **93**, 250 (1947).
[3] Hart, J. F. L., *Canad. J. Res.*, **12**, 411 (1935).

Calcium–Lithium Competition in Absorption by Plant Roots

In the process of absorption by plant roots, different ions may compete with each other, may fail to compete, or finally, one kind of ion may increase the rate of absorption of another. These specificities are considered due to the existence of specific binding sites on carriers which transport the ions across cellular membranes. Examples of mutually competing ions are the pairs potassium–rubidium[1] and calcium–strontium[2]. Neither sodium nor lithium competes readily for the potassium–rubidium sites, at low to moderate concentrations, and magnesium is bound loosely if at all by the sites which transport calcium and strontium.

In work with plants, all reported cases of specific mutual competition among mineral ions have involved pairs of ions closely related chemically. However, calcium severely inhibits the absorption of lithium by barley roots (ref. 3 and Epstein, E., unpublished work), and it has now been determined that the interference is competitive, indicating identical binding sites for these two ions.

Barley, variety Arivat, was grown in dilute calcium sulphate as described earlier[2]. The absorption experiments were conducted with 1-gm. portions of excised roots in 100 ml. volumes of aerated solutions at 30° C., for 60 min., during which absorption was a strictly linear function of time. The concentration of lithium chloride was varied from 1·0 to 10·0 m.moles/l., in the absence and presence of calcium sulphate at 1×10^{-4} and 1×10^{-3} *M*. The roots were rinsed, ashed at 500° C., and lithium in a solution of the ash was determined by flame spectrophotometry at 671 mμ. In Fig. 1, the reciprocal of the rate of absorption, v (in μmoles/gm./hr.), is plotted against the reciprocal of the lithium concentration, S (in m.moles/l.). In the presence of calcium, the slope of the line increases without change of the intercept, indicating competitive interference by calcium with lithium absorption.

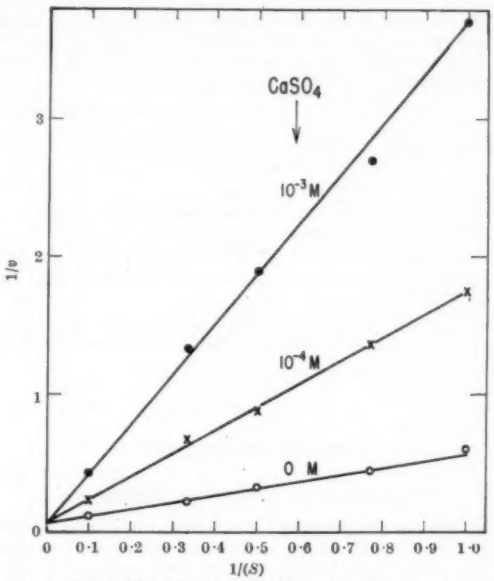

Fig. 1. Competitive inhibition of lithium absorption by calcium

Calcium, which is here shown to inhibit severely and competitively the absorption of lithium, accelerates the absorption of several other ions, among them potassium[4] and rubidium (Table 1). These findings confirm the earlier conclusion[1], recently questioned by Sutcliffe[5], that there are sites transporting lithium which are distinct from others which transport potassium and rubidium, calcium strongly competing with lithium for the former sites while increasing the rate of potassium–rubidium transport.

Table 1. Effect of Calcium on Absorption of Rubidium by Barley Roots

$CaCl_2$, $M \times 10^3$	0	1	2	5	10	25
Rb* uptake, μmole/gm. fresh wt./hr.	8·9	11·8	13·3	14·8	16·0	16·5
$CaSO_4$, $M \times 10^3$	0	1	2	5	10	
Rb* uptake, μmole/gm. fresh wt./hr.	8·9	9·8	11·1	11·0	11·8	

* Rubidium chloride, 1×10^{-3} *M*. Rubidium labelled with rubidium-86, assayed by determination of the radioactivity of the root ash. Experimental conditions and procedures as described in the text for the experiment on $Ca^{++} - Li^{+}$ competition.

At first it seems surprising to find competition between two such dissimilar ions as an alkaline earth cation, calcium, and an alkali cation, lithium. However, it is recognized that the small size and relatively large ionizing potential of lithium cause some resemblance between it and the alkaline earth cations[6]. An instance illustrating this resemblance is the low solubility of the carbonate and phosphate of lithium. Another example concerns the expansion of mica type crystal lattices as it depends on the interlayer cations. Barshad[7] has shown that montmorillonite saturated with lithium is similar in swelling behaviour and differential thermal analysis to samples with alkaline earth interlayer cations, and differs characteristically from samples saturated with the other alkali cations or ammonium ions. Finally, lithium, like calcium, increases the rate of rubidium absorption by barley roots[1].

These similarities seem to reflect ionic properties important in determining the 'fit' of the ions in molecular groupings of ligands. Lithium ions resemble the alkaline earth cations more than the other alkali cations in some of the properties that determine chemical affinities, including affinity for the carrier sites involved in active ion transport.

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[1] Epstein, E., and Hagen, C. E., *Plant Physiol.*, **27**, 457 (1952).
[2] Epstein, E., and Leggett, J. E., *Amer. J. Bot.*, **41**, 785 (1954).
[3] Jacobson, L., Moore, D. P., and Hannapel, R. J., *Plant Physiol.* (in the press).
[4] Overstreet, R., Jacobson, L., and Handley, R., *Plant Physiol.*, **27**, 583 (1952).
[5] Sutcliffe, J. F., *Biol. Rev.*, **34**, 159 (1959).
[6] Latimer, W. M., and Hildebrand, J. H., "Reference Book of Inorganic Chemistry", third ed. (The Macmillan Co., New York, 1951).
[7] Barshad, I., *Amer. Mineralogist*, **35**, 225 (1950).

RADIOBIOLOGY

Resistance of Algae to Radiation

Experiments carried out since 1954 have shown that various algae are much more resistant to radiation than are higher plants and animals. The results, which will be published fully elsewhere, are as follows:

Algae	Author	Dose survived (rads)
Spirogyra	Godward, 1954, and unpub.	20,000 plus
Zygnema, Mougeotia	Prasad, 1959, unpub.	50,000–70,000
Cosmarium	Nizam, 1959, unpub.	70,000
Euglena	Leedale, 1958	150,000
Eudorina	Rayns, 1959, unpub.	100,000
Chlorella	Nizam, 1959, unpub.	two successive doses of 1,000,000
Chaetomorpha	Patel, 1959, unpub.	50,000 plus
Prorocentrum	Dodge, 1959, unpub.	2,000

Of the above, only the last-mentioned, *Prorocentrum*, has the same order of sensitivity as the higher plants, if one excepts their specially resistant parts such as pollen grains and seeds. In all the experiments the algae were maintained in culture as long as possible after radiation; the recovery could follow in a matter of days but might need up to five weeks. Special features of the nuclear cytology probably account for the resistance and rapid recovery of the Conjugales[2,3,5] and *Euglena*[4]. These features do not occur in *Eudorina*, which has, however, very small chromosomes (ref. 1 and Rayns, unpublished work). Smaller still and unresolvable with the visual light microscope except at prophase, and even then uncountable, are those of *Chlorella*, which it seems impossible to kill. On the other hand, the chromosomes of *Prorocentrum* are relatively large, and it would seem that, like those of the higher plants in general, they may have a localized centromere. In those algae where the chromosome organization is conventional or at any rate gross, it seems that resistance to radiation is relatively low. No organism has, to my knowledge, been reported as surviving an experimental dose such as that given above to *Chlorella*, where the recovery process was, however, long; although it seems clear that the marine algae which received considerable radiation from atomic explosions were not killed.

The radiations were carried out at the Medical Research Council Unit, Hammersmith Hospital.

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[1] Cave, M., and Pocock, M. A., *Amer. J. Bot.*, **38**, 800 (1951).
[2] Godward, M. B. E., Proc. Int. Bot. Congr., Paris, 1954; *Heredity*, **11** (1957).
[3] King, G. C., *New Phyt.* (in the press).
[4] Leedale, G. F., *Archiv Mikrobiol.*, **32**, 32 (1958).
[5] Prasad, B. N., thesis, Ph.D., London (1959).

Radiocarbon Dating of Soil Humus

The turn-over of soil organic matter is a much-discussed problem in pedology. Although much has been written about the rate of breakdown of soil organic matter in mull and mor (for references see ref. 1), our knowledge of the turn-over of forest soil humus is very incomplete. This is particularly true of the organic matter in soil horizons below the humus layer.

The radiocarbon dating method opens a way to determine the approximate 'time of residence' in the soil of the organic matter, supposing that this time is of the order some hundred years or more. For this reason three soil samples from a profile in Garpenberg, Province of Dalarna, Middle Sweden, have been studied. The sample plot was a good-growing 95-year old spruce forest on moraine, altitude 200 m. (just above the so-called highest coastline). The samples represented the mor layer (A_0), the leached but humus-coloured top horizon of the mineral soil (A_1), and the brown accumulation horizon (B). The profile, which will be described in more detail in a later publication, is a favourable type of the podzol profile; similar profiles are not uncommon on moraine soils in this region.

The samples were passed through a 3-mm. sieve leaving stones, gravel and all but the finest roots on the sieve. The sifting process passed 63 per cent of the A_0, 90 per cent of the A_1 and 76 per cent of the B layers, based on samples collected in the field (measured dry).

The loss on ignition was 45·5 per cent of the A_0 sample, 15·9 of the A_1 and 5·2 of the B sample. In A_0 and A_1 the loss on ignition can be considered as estimates of the organic matter content, which, however, is lower than 5·2 in the B horizon.

The residual carbon-14 activity was measured with the following results:

Tree rings of oak grown 1845–1855 in Stockholm. Corrected for decay up to 1959	100·0 ± 0·3 per cent
St. 472, Sample A_0	98·3 ± 0·5 "
St. 473, Sample A_1	98·0 ± 0·5 "
St. 474, Sample B	95·6 ± 0·5 "

The activities have been corrected to the same initial isotopic composition by measuring the ratio of carbon-13/carbon-12 in all four cases (see Östlund[2]).

Owing to the well-known decrease of carbon-14 activity in the biosphere between 1850 and 1954 caused by industrial fuel combustion (Suess-effect), it is not possible to draw any reliable conclusion about the time of residence for samples A_0 and A_1. Sample B, however, shows a decrease of activity which is larger than the Suess effect. The calculated value for the time of residence is 370 yr., but the experimental errors give a total time-span of ± 100 yr. (95 per cent probability). In addition, the variations of the carbon-14 assay of the biosphere in times also before 1850 (de Vries[3]) add an extra unknown uncertainty.

Considering the fact that the site sampled probably has been covered with forest for about 9,000 years, the relatively low age of the organic matter in the B horizon suggests that the organic matter in this horizon (or a considerable part of it) is continually broken down and re-synthesized from substances coming from above. This is important, because in this and many other forest soil profiles the amount of organic matter in the B horizon equals or exceeds that in the A horizon. Further investigations seem called for, both by radiocarbon measurements and by other methods.

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[1] Handley, W. R. C., Forestry Comm. Bull., No. 23, 1 (London, 1954).
[2] Östlund, H. G., *Arkiv Kemi*, 12, No. 6, 69 (1957); see p. 74.
[3] De Vries, H. L., *Proc. K. Ned. Akad. Wetensch.*, B, 61, 94 (1958).

Accumulation of Cæsium-137 by Plants grown in Simulated Pond, Wet Meadow and Irrigated Field Environments

Plants grown in nutrient culture[1,2] and aquatic environments[3] accumulate more cæsium-137 than terrestrial plants. The work cited indicates that this isotope should be more available from fall-out under one set of environmental conditions than another. Consequently, the availability of cæsium-137 to plants was examined under conditions simulating shallow ponds, wet meadows and irrigated fields.

The spotted Persicaria or lady's thumb (*Polygonum persicaria* L.), which is tolerant of wide moisture conditions, was transplanted from the field into 7,000 gm. Ephrata Sandy Loam soil[1] in 'Pyrex' battery jars. Stable cæsium was not added to the soil because increased uptake of cæsium-137 with addition of carrier cæsium has been shown[4]. After the plants were well established, 72·3 μc. of $^{137}CsCl_2$ in distilled water was added uniformly to the surface of water or soil, care being taken to avoid contamination of the aerial surfaces.

Plants were grown outdoors under conditions shown in Table 1. There were three pots for each environment and 12 plants per pot. Water-levels in the 'shallow pond' and 'wet meadow' cultures were maintained by adding distilled water with automatic devices while moisture in the 'irrigated field' cultures was maintained by replacing weight loss daily.

Table 1. Concentration of Cæsium-137 in Plants grown in Different Environments

Environment simulated	Experimental condition	Mean value at 5 hr.* (μc./gm.)	Uptake ratio, field capacity = 1	Mean value at 36 days (μc./gm.)	Uptake ratio
Shallow pond	Saturated soil with 2½ in. standing water	$3·1 \times 10^{-3}$ ±0·86	75	$1·4 \times 10^{-2}$ ±0·32	450
Wet meadows	Saturated soil, water table ½ in.	$8·2 \times 10^{-5}$ ±1·40	2	$8·9 \times 10^{-4}$ ±0·92	30
Irrigated field	Soil moistened to field capacity	$4·2 \times 10^{-5}$ ±0·65	1	$3·1 \times 10^{-5}$ ±0·67	1

* Each mean represents nine samples.

Plant stem tips (apical bud, stem and first two leaves) were sampled at intervals over a 36-day period. Samples were wet-ashed in nitric acid at temperatures less than 500° C. Radioactivity of the samples was measured with an end-window Geiger–Müller tube. Results of sampling at 5 hr. and 36 days are shown in Table 1.

The uptake ratio for plants grown in shallow standing water, saturated and field-capacity soils was 450 : 30 : 1. The high uptake in standing water is in close agreement with results obtained with emergent plants in an aquatic community[3], and not appreciably different from nutrient solution studies[2].

The greater uptake of cæsium-137 by plants grown in soil covered by shallow water and saturated soils is due mainly to the shallow root systems produced by plants in such environments, although some absorption by submerged leaves and stems would be expected. These root systems lie close to the surface in saturated soils, and adventitious roots, as in this experiment, often invade the water proper[5–7]. Thus, cæsium-137 can be absorbed directly from the water before the ions are fixed on to the soil. This is supported by results (Table 1, column 3) which show 20 per cent of total accumulation occurred during the first 5 hr.

The radioactivity of plants grown in field-capacity moisture was not appreciably greater than the level in plants taken from the natural habitat. This confirms reports of low availability of cæsium-137 in drier soils[8].

When applied to fall-out, the results reported here may be of considerable interest. Because uptake via roots is additive to direct fall-out on to the aerial plant surfaces, animals grazing in areas in which a major portion of the forage is from wet-lands may ingest cæsium-137 to 400-fold or more over those grazing on dry-lands. Similarly, crops grown in saturated soils or standing water should contain more cæsium-137 than crops grown on drier soils. Thus, increased accumulation of cæsium-137 from wet-lands may explain deviations from a correlation between rainfall and milk content[8,9] and added root absorption may contribute to the high cæsium-137 content of rice in Japan[10]. Further, a few dairy herds, grazing wet or periodically flooded meadows and edges of shallow ponds, might contribute disproportionately to the

cæsium-137 content of a local milk supply, and extensive wet-lands could elevate the contamination-level of a larger region as has been suggested by Russell[11] relative to strontium-90 content of milk in England.

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[1] Rediske, J. H., and Hungate, F. P., Proc. Internat. Conf. Peaceful Uses Atomic Energy, **13**, 354 (1955).
[2] Klechkovsky, V. M. (ed.), Acad. Sci. U.S.S.R. (*A.E.C.-Tr*-2867) (1957).
[3] Pendleton, R. C., and Hanson, W. C., Proc. Second Internat. Conf. Peaceful Uses Atomic Energy, **18**, 419 (1958).
[4] Fredriksson, L., Eriksson, B., Rasmuson, B., Gahne, B., Edvarson, K., and Löw, K., Proc. Second Internat. Conf. Peaceful Uses Atomic Energy, **18**, 449 (1958).
[5] Emerson, F., *Bot. Gaz.*, **72**, 359 (1921).
[6] Dean, B. E., *Plant Physiol.*, **8**, 203 (1933).
[7] Alberda, T., *Plant and Soil*, **5**, 1 (1953).
[8] Booker, D. V., *Nature*, **183**, 921 (1959).
[9] Anderson, Ernest C., *Science*, **128**, 882 (1958).
[10] U.N. Scientific Committee, Supp. No. 17 (*A*/3838), 108 (1958).
[11] Russell, R. Scott, *Nature*, **182**, 834 (1958).

ENTOMOLOGY

Automatic Time/Response Records with *Drosophila*

Drosophila melanogaster Meig. is widely used for bioassay of insecticides. In developing a method for bioassay of insecticide residues in soil, we have found it necessary to use a time/response technique similar in principle to that employed by Callaway, Dirnhuber and Wilson[1]. In comparison with the more usual dose/response techniques, the sensitivity[2] and also the useful range of the assay can be increased by this means. However, with a time/response technique, two main difficulties arise when attempts are made to obtain records at precise times in order to follow accurately a given programme of observations. The response of each group of flies takes a finite time to assess and so, when many groups are involved, discrepancies occur between the desired and the actual times of the counts. Unless allowances are made for these time discrepancies—a tedious process—errors are introduced which can be important when the intervals between the records are small. Furthermore, it is often necessary to record responses throughout a 24-hr. period, or even longer, and this creates an obvious practical problem which, in the past, must have discouraged the use of time/response techniques as routine methods.

A brief description is given below of an apparatus which we have devised to record automatically the response of *Drosophila* at any number of pre-arranged times. The apparatus has worked successfully in this laboratory for the past six months and a detailed description of it is being prepared for publication elsewhere.

Acetone is used to extract the insecticide from the soil. Aliquots of the extracts are applied to 38 × 10 mm. absorbent cotton rolls, and the solvent is allowed to evaporate. A dilute solution of a syrup is added to each roll to provide food for, and so attract, the flies. The treated rolls are suspended in special 130 × 25 mm. 'exposure tubes' and 30 male *Drosophila* are put into each tube. The top of each tube is closed with muslin, but the bottom of the tube tapers evenly towards a 6-mm. diameter exit hole. A ventilation hole, 10 mm. in diameter, is present in the side-wall 30 mm. from the bottom of the tube. Thirty of these tubes are mounted vertically in a rack. In the base of the rack there are countersunk 6-mm. diameter holes into which fit the tapered bottoms of the exposure tubes. These holes are normally kept closed by a sliding shutter which has a complementary set of holes and is fitted underneath the base of the rack. The shutter is linked mechanically to two solenoids which open and close it in response to signals from a special timing device. The rack carrying the tubes is mounted immediately over a 152-mm. diameter cylinder which is coated with a non-setting gum and powered by an internal coiled spring. This can be wound up by a crank arm attached to a stub-axle passing through the end-plate of the cylinder. A pin-escapement, fitted externally to the other end-plate, limits the movement of the cylinder and is also operated by a solenoid activated by a signal from the timer.

The time of each operation of the apparatus is determined by an accurately driven tape in which holes have been punched at intervals to correspond with the required time sequence. An electrical contact detects each hole as it passes and the three signals required to work the solenoids of the apparatus are initiated. There is first a short pulse to open the shutter, followed immediately by a second, slightly longer pulse to close it and then, after a delay of about 5 sec., a third pulse causes the cylinder escapement to be released.

The timer is started as soon as flies have been put into half of the exposure tubes, which takes about 2 min., and, as the flies become affected by the insecti-

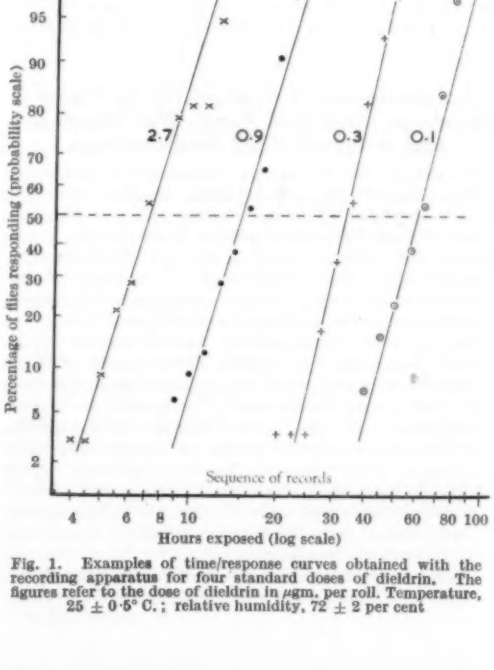

Fig. 1. Examples of time/response curves obtained with the recording apparatus for four standard doses of dieldrin. The figures refer to the dose of dieldrin in μgm. per roll. Temperature, 25 ± 0·5° C.; relative humidity, 72 ± 2 per cent

cide, they fall and collect in the recesses formed by the holes in the base of the rack. The apparatus is illuminated from above so that unaffected flies are attracted upwards and away from the exit holes. At an appropriate time, the shutter moves and opens the exit holes momentarily so that the affected flies fall out on to the sticky surface of the cylinder, where they are retained in discrete groups. The escapement is then released and allows the cylinder to move 1/16 of a revolution into position for the next record. At suitable times the number of flies in each position on the cylinder is noted, and the frequency results so obtained enable the time for 50 per cent response to be estimated in the usual way for each dose. The time/response curves shown in Fig. 1 are examples of results with this apparatus.

The programme of observations should be chosen so that there are equal grouping intervals for response to different doses and thus the programme used depends on the relationship between time of exposure and response to any given dose. Under our experimental conditions :

$$Y = a + b \log t$$

where Y is the probit of the percentage of flies responding to any given dose at time t, a is a function of dose in any one assay and b is the regression coefficient (Fig. 1). This relationship is that usually found when exposure to the poison is continuous[1,3,4] and it was thus necessary to arrange for observations to be made at equal intervals on a logarithmic, and not linear, time-scale ; the tape was therefore programmed accordingly.

We wish to thank Mr. J. A. Nelder for numerous fruitful discussions while the apparatus was in the design stage, and also Mr. D. W. Wright for the help and encouragement which he has given throughout the work.

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[1] Callaway, S., Dirnhuber, P., and Wilson, K. M., *Nature*, **170**, 843 (1952).
[2] Bartlett, B. R., *J. Econ. Ent.*, **44**, 621 (1951).
[3] Bliss, C. I., *Ann. App. Biol.*, **24**, 815 (1937).
[4] Bliss, C. I., *Ann. Ent. Soc. Amer.*, **33**, 721 (1940).

Physiological Age of Codling Moth Females (*Cydia pomonella* (L.)) caught in Bait and Light Traps

Several recent papers have compared the effectiveness of bait and light traps as means of estimating changes in adult codling moth populations and of timing the application of sprays[1–3]. The comparison of these trapping devices was based, in each instance, on the total number of moths caught over periods considered to have been critical for the subsequent infestation of the crop. In these investigations no attention was paid to the quality of the respective catches beyond noting the difference in the relative frequency of females attracted to each type of trap.

When trap catches are used for recording moth activity as a guide to timing spray application, the age of females in samples may be of little importance if the weather conditions impose a fairly long developmental period on eggs. However, the physiological age of the trapped moths must be carefully considered when interpreting catch results obtained under warmer conditions. This point was emphasized by Nel[4], whose investigations showed that bait trap samples consist predominantly of spent, or half-spent, females.

Some additional information has been gained in the course of an experiment on the performance of bait and light traps carried out in an apple orchard near Canberra, Australia. Four bait traps and one light trap were operated daily in alternating positions from November 1958 until March 1959. A standard lure of fermenting molasses incorporating pine-tar oil[5] was used in the bait traps. The light source was an *HP* 125 W.B.C. mercury vapour lamp. Females obtained from both types of traps throughout the season were fixed and stained according to the method of Hamstead and Gould[6]. Each female was dissected, and an estimate of its physiological age made in strict conformity with Nel's techniques and criteria.

The age distribution of the females caught in bait traps at Canberra was found to be almost identical with that observed by Nel in South Africa. It is thus likely that most females are not attracted to the bait before some of their first-laid eggs have had time to hatch.

Table 1

	Physiological age I (young)	Physiological age II (middle-aged)	Physiological age III (old)	Total
Bait traps	14	39	36	89
Light trap	113	74	11	198

$\chi^2 = 70_{(2)}$ $P < 0{\cdot}001$

The picture is entirely different for the light trap, which drew samples of quite significantly younger females from the field population (Table 1). This feature may be added to the previously recognized advantages of light traps over bait pans in codling moth population studies.

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[1] Groves, J. R., Ann. Rep. East Malling Res. Sta., 1954, **146** (1955).
[2] Savary, A., and Baggiolini, M., *Ann. Agric. Suisse*, **56**, 827 (1955).
[3] Schneider, F., Vogel, W., and Wildbolz, T., *Schweiz. Z. Obst.- u. Weinb.*, **66**, 410 (1957).
[4] Nel, R. I., *Ent. Memoirs*, **2**, 55 (1940).
[5] Van Leeuwen, E. R., *J. Econ. Ent.*, **36**, 430 (1943).
[6] Hamstead, E. O., and Gould, E., *J. Econ. Ent.*, **43**, 724 (1950).

ANATOMY

A Highly Organized Structure within a Neurosecretory Vesicle

Previous electron microscope studies have demonstrated an abundance of vesicles 1000–3000 Å. in diameter in the terminal branches of neurosecretory axons in the neurohypophysis[1], and in the sinus glands and pericardial organs of crustaceans[2,3]. I have recently observed that some of these neurosecretory vesicles possess a highly organized internal structure.

The pericardial organs of the Mediterranean stomatopod *Squilla mantis* were prepared for electron microscope study by fixation either in buffered

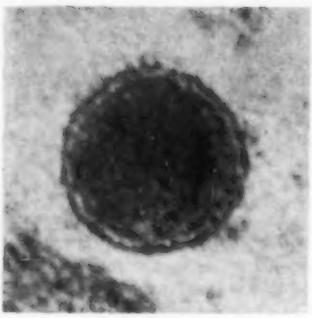

Fig. 1. A single neurosecretory vesicle of type *A* (× 225,000). Material fixed in buffered potassium permanganate, embedded in 'Araldite'

osmium tetroxide or buffered potassium permanganate and subsequent embedding in 'Vestopal *W*' or 'Araldite'.

In these preparations two types of vesicle could be clearly distinguished by their size and electron density and will be referred to here as types *A* and *B*.

Type *A* vesicles are regular in outline, spherical or slightly ovoid, approximately 1500 Å. in diameter. Each is surrounded by a distinct unit membrane, 75–90 Å. in thickness. The contents of type *A* vesicle can only be distinguished with difficulty as they are very electron dense. They appear to consist of tightly packed units without geometrical order, each unit comprising an electron-dense particle approximately 50 Å. in diameter, surrounded by a less dense sheath some 75 Å. in thickness (Fig. 1).

Type *B* vesicles, about 1200 Å. in diameter, are generally irregular in outline, though approximately spherical or ovoid; the bounding membrane is similar to that enveloping the type *A* vesicle, though less distinct, and there is usually a clear space between the membrane and the contents of the vesicle. The type of order displayed by the contents of the vesicle in various projections demonstrated in the electron micrographs is such as to suggest that the organization

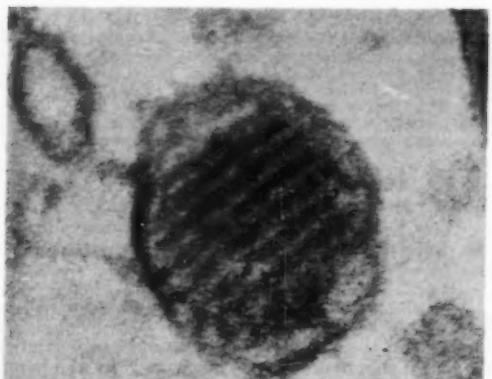

Fig. 2. A single neurosecretory vesicle of type *B* and two smaller vesicles (× 310,000). The orderly arrangement of material in the larger vesicle may be seen, and also a connecting link between the larger and one of the smaller vesicles. Material fixed in buffered osmium tetroxide, embedded in 'Vestopal *W*'

is based on a precise hexagonal packing of rod-shaped components. In the projection shown in Fig. 2 the structure appears as a series of alternating light and dark bands each measuring 60 Å. in width.

Fibres containing type *B* vesicles also contain smaller vesicles, spherical, ovoid or elongate in profile, approximately 500 Å. in diameter. There are indications that these small vesicles form part of a continuous tubular system in the axoplasm and that the type *B* vesicles are intimately related to this system. Indeed, it seems possible that the material in the type *B* vesicle may form within the system of small vesicles, and that the type *B* vesicles are swollen parts of this system within which material has assumed a crystal-like order.

The detection of orderly differentiated systems within neurosecretory particles is of theoretical interest, for there have been some indications from histochemistry also that neurosecretory vesicles may contain at least two chemical components[4]. The type of organization indicated by the present electron micrographs may, in fact, represent an orderly arrangement of these components which might permit the maximum of interaction between them in a suitable milieu.

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[1] Palay, S. L., *Anat. Rec.*, **121**, 348 (1955).
[2] Hodge, M. H., and Chapman, G. B., *J. Biophys. Biochem. Cytol.*, **4**, 571 (1958).
[3] Knowles, F. G. W., "Comparative Endocrinology", 223 (John Wiley, 1959).
[4] Hild, W., and Zetler, G., *Pflüg. Arch. ges. Physiol.*, **257**, 169 (1953).

'Synaptic Vesicles' in the Neurohypophysis

Electron microscope studies of the neurohypophysis have shown that the terminal branches of the hypothalamo-hypophysial tract fibres contain, in addition to mitochondria, vesicles or granules of two kinds distinguished by their size[1,2]. The larger inclusions each have a diameter of the order of 100–300 mμ (1000–3000 Å.), and are thought to represent the secretory material which may be demonstrated by histochemical means, using the light microscope. The smaller inclusions, with diameters of approximately 30 mμ (300 Å.), have been described as synaptic vesicles[1] because they resemble in size inclusions found elsewhere in the nervous system at nerve terminals.

In the course of studying the neurohypophysis of rats and ferrets with the electron microscope, using material fixed either in buffered osmium tetroxide or in buffered potassium permanganate, we have observed a correlation of vesicle size with fibre size and fibre position. Small fibres, especially those closely applied to capillary walls, generally contain small vesicles, often densely packed and filling the whole outline of the fibre; in such fibres the larger vesicles are either absent or few in number (Figs. 1 and 2). In larger fibres the larger vesicles are found, either alone or with some small vesicles also. Large fibres containing small vesicles only have not been observed.

The correlation of vesicle size and fibre size is in itself not inconsistent with the view that the smaller vesicles are analogous to vesicles found at synaptic terminals. It is significant, however, that when large vesicles are found in fibres closely adjacent to capil-

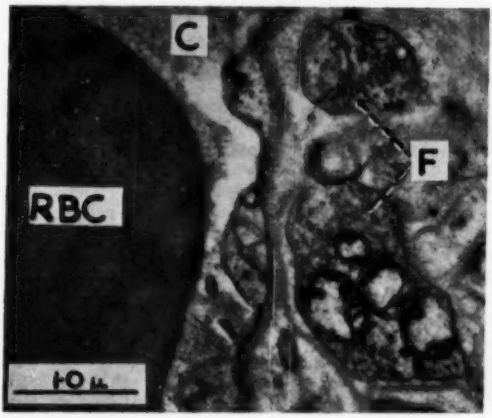

Fig. 1. A small portion of the neurohypophysis of a rat in which neurosecretory fibres (*F*) in close proximity to a blood capillary (*C*) are shown, and are seen to contain only small vesicles and mitochondria. *RBC*, part of a red blood cell. Fixed in buffered potassium permanganate, embedded in 'Araldite'

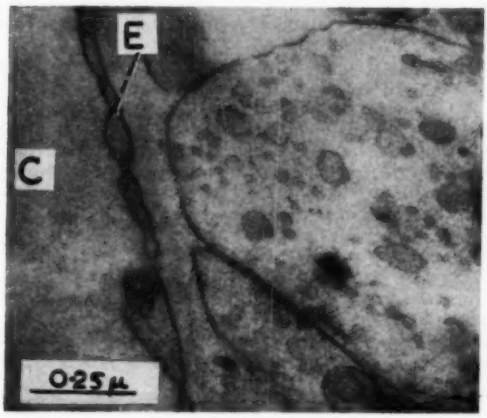

Fig. 2. A similar region in the neurohypophysis of a ferret. Indications of breakdown of large vesicles to small are apparent. *E*, fenestrated endothelium. Fixed in buffered potassium permanganate, embedded in 'Araldite'

laries, an internal structure may be detected in them which indicates that they may contain an aggregation of inclusions of the smaller type. There is, therefore, an interesting parallel between the vesicles in the nerve terminations of the mammalian neurohypophysis, and those in the pericardial organs of crustaceans, in which an orderly arrangement of small vesicles or particles within larger vesicles has been detected (previous communication).

As a result of these findings, we believe that it is imprudent at present to imply[1], by the use of the same term, that all small vesicles in the terminations of neurosecretory fibres resemble functionally the synaptic vesicles found elsewhere in the nervous system.

R. L. Holmes
F. G. W. Knowles

Department of Anatomy,
University of Birmingham.

[1] Palay, S. L., *Anat. Rec.*, **121**, 348 (1955).
[2] Hartmann, J. F., *Z. Zellforsch.*, **48**, 291 (1958).

ARCHÆOLOGY

A New Palæolithic Find in Tapti Valley, Khandesh, India

In 1863 Foote reported a first palæolith from laterite gravels occurring at Pallavaram, near Madras. This find proved the presence of early man, maybe in the Pithecanthropoid or Neanderthaloid stage in India[1].

De Terra and Paterson[2] later recognized different phases of glacial cycle in Narbada valley. They were able to point out that early Palæolithic 'flake industries' were associated with Middle Pleistocene *Elephas antiqus* and *E. namadicus* and that three different cycles of sedimentation could be distinguished and correlated with the three stages in the formation of Narbada alluvium.

Pierre Teilhard de Chardin[3] of the Wenner–Gren Foundation, New York, points out in his good-will message to the Palæontological Society that ". . . first care should be taken to organize with the help of a few trained collectors, some kind of permanent surveyance : of the Narbada Basin (for the research of the hand-axes Man of India, skull or limb-bones. . .). Between S.E. Africa and Java, India happens to hold an exceptionally critical place, as far as the origin of Man is concerned".

During geological and ecological surveys of Khandesh Valley, I came across an interesting spot beside the Girna River bank near Central Railway bridge, 3 miles from Jalgaon, situated 21° 1′ N. and 75° 35′ E., 261 miles north-east of Bombay.

The River Girna rises in the hills west of Kalyan, in Nasik district. It is fed by streams from the northern slopes of the Sapta-Shring Range. After a course of about 150 miles, it joins the Tapti near Nander. Its course lies in equal parts in Nasik and Khandesh. Passing through Nasik almost in a straight line eastwards, its course changes to north-east in Khandesh. Near Jalgaon, it bends north and north-west, flowing for several miles with many windings almost parallel to the Tapti.

The underlying rock formation at places is Deccan trap. On the banks of the River Girna pebble conglomerate abound. They are capped by layers of sandy alluvium. This indicates that the river must have overflowed at times. The river now appears to be eroding ancient beds and underlying rocks.

Fig. 1. Scrapers from the Girna River banks

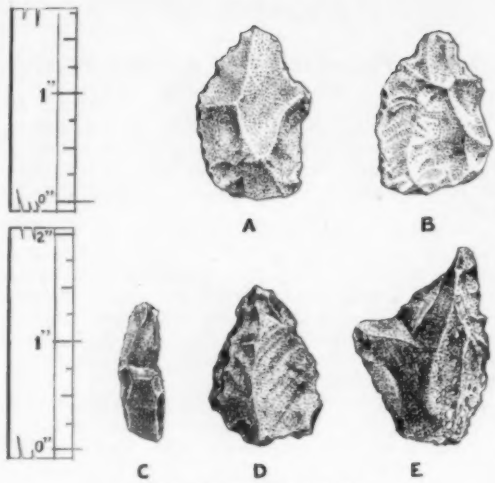

Fig. 2. Scrapers (dorsal view)

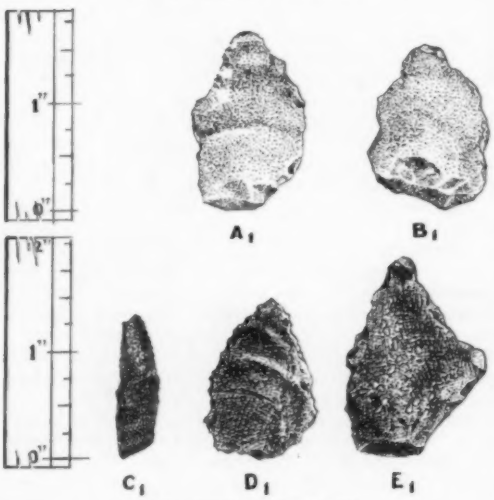

Fig. 3. Scrapers (ventral view)

The specimens of flakes could be of upper Acheulian tools belonging to early Sohan industries[2]. The scrapers show resemblances with early Soan flakes.

To my knowledge there is no record of such palæolithic find hitherto from Khandesh, this being the first find of such type from the Girna River in the Tapti Valley from Khandesh. It is suggested that a systematic survey of the river valleys which has hitherto been neglected be undertaken by archæologists, as it may one day show rich finds since this area was once a Narbada basin during the post-Eocene era.

C. R. Karnik

Mooljee Jaitha College,
Jalgaon,
East Khandesh,
India.
Nov. 15.

[1] Foote, J., *J. Palaeo. Soc. India*, 1 (1), 28 (1956).
[2] De Terra and Paterson, "Studies in the Ice Age in India and Associated Human Cultures" (Washington, 1939).
[3] De Terra and Paterson, *J. Palaeo. Soc. India*, 1 (1) (1956).

EDUCATION

"'Teen-Age' Education in England"

You have, of course, a perfect right to say (*Nature*, February 6, 1960) that, in your opinion, the Crowther Report has made a wrong choice of priorities, but have you the right to leave out of your article any reference to the conditions that that Report lays down as essential if the age is to be raised? It calculates the number of extra teachers that will be needed not only to cover the extra year, but also at the same time to reduce over-large classes. It concludes that the task of providing these teachers, though formidable, is not beyond the capacity of the country, if it is determined to accept the plan. With regard to curriculum, it says that a greater variety of courses must be provided, that methods of teaching and discipline should be altered so that the fifteens–sixteens will be treated more like adults and less like schoolchildren, and that staffing ratios should be generous enough to allow "for some adult leadership out of school hours".

Readers of the article in *Nature* who have not read the Report itself would not know that it contemplates these changes before the age can be raised.

With regard to the note by what is called a "strong minority of the Committee", which did not, incidentally, include any of the heads of secondary modern schools, this dealt with the treatment of a small minority of children. The signatories go out of their way to state: "To avoid any possibility of misunderstanding, we want to make it clear that this note does not imply any dissent on our part from the major recommendation that the school-leaving age should be raised. On the contrary, we feel confident that provision on the lines we have suggested of a small minority would be held in public opinion to reaffirm the case for keeping the great majority in school for another year. We certainly regard it ourselves." Apparently, they have not avoided misunderstanding on your part.

It is stated in *Nature* that "No member of the Crowther Committee teaches the lower streams of Modern Schools". This is equivalent to the charge that the three heads of secondary modern and one head of a comprehensive school are ignorant of the conditions which exist in their schools—a grave indictment indeed that would have to be supported with strong evidence.

Since many States in the United States of America have had a school-leaving age of sixteen for more than twenty years, and some, one of 17+, why should we assume that teachers in England are not able to make a sixteen leaving age "socially acceptable" if their counterparts across the Atlantic have already done so? Teachers in England have always responded to the increasing demands that we have made upon them, and I, for one, refuse to believe that they will fail the nation now, provided that we give them the right conditions for work.

Shena D. Simon

Broomcroft,
Ford Lane,
Didsbury,
Manchester 20.

FORTHCOMING EVENTS

(Meetings marked with an asterisk • are open to the public)

Monday, March 7

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Dr. J. C. White: "Abnormal Hæmoglobins".•

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. A. T. A. Learmonth: "Medical Geography in India and Pakistan".

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. Arthur Koestler: "Some Aspects of the Psychology of Discovery".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. P. N. Butcher: "An Introduction to the Theory of Masers with particular reference to the Travelling-Wave Maser".

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. R. Lenoir: "Progress of British High Grade Watch Production".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. Bernard O. Phinney (University of California): "The Use of Biochemical Genetics in the Study of Gibberellin-controlled Growth".•

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. W. Gerrard: "Recent Advances in the Chemistry and Industrial Applications of Hydrido- and Organo-Boron Compounds".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. A. S. Younger, Mr. G. C. Morgan and Mr. E. S. Mallett: "Data Reduction for Guided Weapon Trials at Aberporth".

Tuesday, March 8

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. W. B. Emery: "A Middle Kingdon Fortress in the Sudan".•

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Dr. F. Sanger: "Amino Acid Sequences in Proteins".•

BRITISH GLACIOLOGICAL SOCIETY (at Bedford College, Regent's Park, London, N.W.1), at 5.30 p.m.—Annual General Meeting. Mr. H. Lister: "Drifting Snow".

INSTITUTE OF MARINE ENGINEERS (at the Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Cdr. R. G. J. Peaver, R.N.: "Distillation for Marine Purposes".

UNIVERSITY OF LONDON (at Senate House, Malet Street, London, W.C.1), at 5.30 p.m.—Prof. C. G. Hempel (Princeton University): "Some Problems in the Logic of Probabilistic Explanation".•

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. J. W. Granville: "Electronic Microcircuits".

Wednesday, March 9

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. J. E. Richards: "Nuclear Power for Marine Propulsion".

INSTITUTION OF ELECTRICAL ENGINEERS, SUPPLY SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. B. Salvage and Mr. J. A. M. Gibbons: "The Impulse Strength of Impregnated-Paper Dielectrics as used in High-Voltage Cables".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. Bernard O. Phinney (University of California): "Gibberellins as Native Growth Regulators of Flowering Plants".•

INSTITUTE OF INFORMATION SCIENTISTS (at the College of Preceptors, 2 Bloomsbury Square, London, W.C.1), at 6.15 p.m.—Prof. Colin Cherry: "The Nature of Human Communication".

SOCIETY OF CHEMICAL INDUSTRY. FOOD GROUP (joint meeting with the ASSOCIATION OF PUBLIC ANALYSTS, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Scientific Papers.

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7.30 p.m.—Mr. W. J. Egginton: "The Hovercraft and Its Development".

Thursday, March 10

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. A. L. Hales: "Bernard Price Institute of Geophysical Research".

INSTITUTION OF ELECTRICAL ENGINEERS, UTILIZATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. J. P. McBreen: "Some Considerations in the Application of Power Rectifiers and Convertors".

PHYSICAL SOCIETY, ACOUSTICS GROUP (in the Physics Department, Imperial College, Imperial Institute Road, South Kensington, London, S.W.7), at 5.30 p.m.—Dr. J. M. Bowsher: "Electronic Music".

UNIVERSITY OF LONDON (at Senate House, University of London, London, W.C.1), at 5.30 p.m.—Prof. A. Hanau (University of Göttingen): "Some Results of German Research in Agricultural Marketing and Prices".•

CHEMICAL SOCIETY (in the Large Chemistry Lecture Theatre, Imperial College of Science and Technology, South Kensington, London, S.W.7), at 7.30 p.m.—Prof. A. R. J. P. Ubbelohde, F.R.S.: "Ionic Crystals and their Melts" (Liversidge Lecture).

Friday, March 11

ROYAL AERONAUTICAL SOCIETY (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 9.45 a.m.—Symposium on "Vehicle and Instrumentation Problems in Upper Atmosphere Research". Speakers: Prof. Sir H. S. W. Massey, F.R.S., Mr. J. F. Hazell, Dr. R. L. F. Boyd, Dr. G. D. Robinson, Mr. H. J. Higgs and Mr. E. C. Cornford.

PHYSICAL SOCIETY, LOW TEMPERATURE GROUP (at the Institute of Physics, 47 Belgrave Square, London, S.W.1), at 10 a.m.—Symposium on "Measurement Techniques (Flow, Pressure, Level, Temperature, etc.)".

ASSOCIATION OF APPLIED BIOLOGISTS (joint meeting with the BRITISH ECOLOGICAL SOCIETY, at the British Museum (Natural History), South Kensington, London, S.W.7), at 10.50 a.m.—Meeting on "Ecological Effects of Crop Production".

INSTITUTE OF PHYSICS, NON-DESTRUCTIVE TESTING GROUP (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Dr. R. Jones: "Analysis of Road Constructions".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. A. E. Werner: "Scientific Techniques in Art and Archæology".

Saturday, March 12

BRITISH MYCOLOGICAL SOCIETY (in the Botany Department, Birkbeck College, Malet Street, London, W.C.1), from 2.15 p.m. to 5 p.m.—Conversazione.

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss A. G. C. Grandison: "Collecting Reptiles and Amphibians in the United States of America".•

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER (with special qualifications or interests in radio astronomy) IN PHYSICS—The Registrar, The University, Manchester 13 (March 12).

ASSISTANT LECTURER (with special interest in urban geography) IN GEOGRAPHY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 14).

JUNIOR LECTURER (male or female, with an honours degree in chemistry or biochemistry, or A.R.I.C.) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY—The Secretary, Westminster Medical School (University of London), Horseferry Road, London, S.W.1 (March 14).

DEVELOPMENTAL PHYSIOLOGIST (with a good honours degree and preferably two or more years postgraduate research experience) IN THE PLANT PHYSIOLOGY UNIT, Department of Scientific and Industrial Research, Palmerston North, New Zealand, initially to assist in current studies using controlled climate facilities for investigation of the influence of the climatic factors on reproduction in plants of economic importance—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. No. B.11/21/26 and mentioning *Nature* (March 18).

ASSISTANT LECTURER or LECTURER IN BOTANY—The Secretary, The University, Exeter (March 19).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, PHYSICS, ENGINEERING (including CHEMICAL ENGINEERING), METALLURGY, PHARMACOLOGY, or related subjects—The Registrar, The University, Sheffield 10 (March 19).

LECTURER IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, The University, Leicester (March 19).

RESEARCH FELLOW (engineer, physicist or mathematician) IN PRODUCTION ENGINEERING—The Registrar, The University, Nottingham (March 19).

LECTURER or ASSISTANT LECTURER (preferably with experience or interest in magnetic resonance) IN PHYSICS—The Registrar, University College of North Staffordshire, Keele, Staffs (March 21).

LECTURER or ASSISTANT LECTURER (with a good honours degree) IN ORGANIC CHEMISTRY—The Registrar, King's College (University of London), Strand, London, W.C.2 (March 21).

RESEARCH ASSISTANT (graduate, with botany to the standard of a B.Sc. (General) degree) to the Head of the DEPARTMENT OF BOTANY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (March 21).

ASSISTANT LIBRARIAN (woman graduate)—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (March 22).

DEMONSTRATOR IN THE DEPARTMENT OF GEOLOGY, to act as Curator of Collections—The Registrar, The University, Liverpool, quoting Ref. CV/T (March 25).

LECTURER IN AGRICULTURE (ANIMAL BREEDING)—The Registrar, King's College (University of Durham), Newcastle upon Tyne 1 (March 25).

ASSISTANT LECTURER IN INORGANIC CHEMISTRY; and DEMONSTRATORS (2) IN INORGANIC CHEMISTRY—The Registrar, The University, Nottingham (March 26).

ASSISTANT LECTURER IN THE DEPARTMENT OF CHEMISTRY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (March 26).

CHAIR OF METALLURGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (March 26).

LECTURER (with a good honours degree in geology and/or chemistry and research experience in geochemistry) IN GEOCHEMISTRY—The Secretary, The University, 38 North Bailey, Durham (March 26).

LECTURER (with an honours degree or equivalent) IN THE DEPARTMENT OF FLUID MECHANICS—The Registrar, The University, Liverpool, quoting Ref. CV/T (March 26).

SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree in science or engineering and at least three years postgraduate experience) at the Headquarters of the Department of Scientific and Industrial Research, London, S.W.1, for scientific administrative duties—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting E.93/0/A (March 28).

ANALYTICAL CHEMIST (preferably with at least an honours degree with chemistry as a major subject, or a professional diploma of a recognized Chartered Institute) IN THE GOVERNMENT ANALYSTS LABORATORY, Salisbury, Federation of Rhodesia and Nyasaland—The Secretary (R), Rhodesia House, 429 Strand, London, W.C.2 (March 31).

ASSISTANT LECTURER (with an honours degree in botany or agricultural botany, and preferably with research experience and an interest in plant physiology) IN AGRICULTURAL BOTANY—The Registrar, The University, Leeds 2 (March 31).

ASSISTANTS (2) (with a good honours degree in geography and preferably with a special interest in physical geography and/or cartography) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, The University, Edinburgh (March 31).

LECTURER IN THE DEPARTMENT OF PATHOLOGY—The Secretary, The University, Edinburgh (March 31).

PLANT BREEDER AND GENETICIST IN THE DEPARTMENT OF AGRONOMY (PLANT BREEDING SECTION), Waite Agricultural Research Institute, University of Adelaide, Australia, to undertake research on the factors affecting quality and yield in barley and to produce commercial barley varieties—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, March 31).

RESEARCH FELLOW (physicist, physical chemist or electrical engineer) IN GAS DISCHARGES, to work in association with Prof. G. Porter on an investigation of radiation from gas discharges—The Registrar, The University, Sheffield 10 (March 31).

LECTURER (preferably with special qualifications in engineering geology, including hydrology and soil science) IN THE DEPARTMENT OF GEOLOGY, University of the Witwatersrand, Johannesburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, April 15).

LECTURER (graduate in science) IN MICROBIOLOGY—The Secretary, The Queen's University, Belfast (April 30).

RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY, for a research programme devoted mainly to investigating, in collaboration with Dr. W. P. Doyle, the ultra-violet absorption of thin films of inorganic solids—Prof. T. S. Wheeler, Department of Chemistry, University College, Dublin (May 1).

ASSISTANT or ASSOCIATE PROFESSOR OF MATHEMATICS—The Principal, Victoria College, Victoria, B.C., Canada.

ASSISTANT or ASSOCIATE PROFESSOR (with the degree of D.Sc. or Ph.D. in zoology from a recognized university and special interests in the physiology of aquatic organisms, particularly fishes) IN THE DEPARTMENT OF ZOOLOGY—The Chairman, Department of Zoology, University of Manitoba, Winnipeg 9, Manitoba, Canada.

BIOCHEMIST (Basic grade) IN THE PATHOLOGY LABORATORY, to assist the Senior Biochemist—The Superintendent, Liverpool Royal Infirmary, Pembroke Place, Liverpool 1.

REGIONAL TRIALS OFFICER (graduate in agriculture), for agricultural variety trials conducted by the National Institute of Agricultural Botany at the N.A.A.S. Experimental Husbandry Farm, Terrington St. Clement, Norfolk; and an ASSISTANT TRIALS OFFICER (preferably with practical experience in crop production), to work at Leeds University Farm, Headley Hall, Yorks—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER, PHYSICAL CHEMIST or METALLURGIST (with a first- or second-class honours degree or equivalent) at the National Physical Laboratory, Teddington, Middlesex, for work in the Metallurgy Division on the use of radioactive isotopes as tracers in metallurgical investigations—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. F.648/9A.

TECHNICIAN (preferably with some knowledge of physics, chemistry and biology, and experience in photography and section cutting) IN THE ELECTRON MICROSCOPE DEPARTMENT—The Secretary, Strangeways Research Laboratory, Wort's Causeway, Cambridge.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Society for Analytical Chemistry. Report of the Analytical Methods Committee, 1958. Pp. vi+24. (London: Society for Analytical Chemistry, 1959.) [2212

London County Council: Public Health Department—Scientific Branch. Annual Report of the Scientific Adviser for the year 1958. Pp. 42. (No. 4056.) (London: L.C.C., Public Health Department, 1959.) 1*s.* 3*d.* [2212

Imperial College of Science and Technology (University of London). Fifty-second Annual Report of the Governing Body, 1958–59. Pp. ii+102. (London: Imperial College of Science and Technology, University of London, 1959.) [2212

Royal Greenwich Observatory Bulletins. No. 16: Time Service 1959, April–June. Pp. 241–258. (London: H.M. Stationery Office, 1959.) 3*s.* 6*d.* net. [2212

Bowaters in North America. By Robert Sinclair. Pp. 48. (London: The Bowater Paper Corporation, Ltd., 1959.) [2212

Memoirs of the Geological Survey of Great Britain. Palæontology, Vol. 4, Part 2: Fossil Plants of the Carboniferous Rocks of Great Britain. By Dr. Robert Crookall. Second edition. Pp. i–iv+85–216+plates 25–58. (London: H.M. Stationery Office, 1959.) 110*s.* net. [2212

Operator Training in Industry. By W. Douglas Seymour. Pp. 52. (London: Institute of Personnel Management, 1959.) 7*s.* 6*d.* [3012

Other Countries

Population Reference Bureau, Inc., Washington, D.C. Population Bulletin, Vol. 15, No. 7: Japan's Population Miracle: an Asian Solution to Asia's Greatest Problem. Pp. 117–136. (Washington, D.C.: Population Reference Bureau, Inc., 1959.) [3012

Osmania University, Hyderabad. Publications of the Nizamiah Observatory. Vol. 8, part 2: Comparison of the Places of Stars in the "Catalogue of 8824 Stars," Washington, with the Photographic Measures on the Plates of the Hyderabad Section of the Astrographic Catalogue. Under the direction of A. Ali. Pp. ii+27. Rs. 4; 6*s.* net. Spectroheliographic Observations of Solar Flocculi made at the Nizamiah Observatory, Hyderabad, during 1951–1955, No. 1. By H. Alvi. Pp. 11. (Hyderabad: Osmania University Press, 1958 and 1959.) [3012

Government of India: Ministry of Health. Annual Report of the Central Drugs Laboratory from 1st April, 1955 to 31st March, 1956. Pp. i+46. Rs. 5.50; 8*s.* 6*d.* Report of the Central Drugs Laboratory, 1st April, 1956 to 31st March, 1957. Pp. ii+47. Rs. 5.75; 9*s.* (Delhi: Manager of Publications, 1959.) [3012

Commonwealth of Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report—Division of Electrotechnology, 1958–59. Pp. ii+21+9 plates. (Chippendale, N.S.W.: Division of Electrotechnology, National Standards Laboratory, 1959.) [3012

Population Reference Bureau, Inc., Washington, D.C. Population Bulletin, Vol. 15, No. 8: The New Awakening: Symposium of Official Opinion. Pp. 137–160. (Washington, D.C.: Population Reference Bureau, Inc., 1959.) [3012

Smithsonian Institution: Bureau of American Ethnology. Bulletin 143: Handbook of South American Indians, Vol. 7: Index. Pp. vi+286. (Washington, D.C.: Government Printing Office, 1959.) 2 dollars. [3012

National Science Foundation. Federal Funds for Science 8: The Federal Research and Development Budget, Fiscal Years 1958, 1959, and 1960. Pp. iii+74. (NSF–59–40.) (Washington, D.C.: Government Printing Office, 1959.) 45 cents. [3012

United States Department of the Interior: Fish and Wildlife Service. North American Fauna No. 61: Fauna of the Aleutian Islands and Alaska Peninsula. By O. J. Murie. Invertebrates and Fishes collected in the Aleutians, 1936–38. By Victor B. Scheffer. Pp. xiv+406. (Washington, D.C.: Government Printing Office, 1959.) 1.25 dollars. [3012

The Report of the President of the Carnegie Institution of Washington, 1958–1959. Pp. 54. (Reprinted from *Carnegie Institution of Washington Year Book* 58 for the year 1958–1959. Pages 1–54.) (Washington, D.C.: Carnegie Institution of Washington, 1959.) [3012

World Health Organization. Technical Report Series, No. 183: Mental Health Problems of Automation—Report of a Study Group. Pp. 30. (Geneva: World Health Organization; London: H.M. Stationery Office, 1959.) 1 Swiss franc; 1*s.* 9*d*; 0.30 dollars. [3012

United States Department of the Interior: Fish and Wildlife Service. Fishery Bulletin 157: The Goldeye, *Amphiodon alosoides* (Rafinesque), in the Commercial Fishery of the Red Lakes, Minnesota. By Marvin D. Grosslein and Lloyd L. Smith, Jr. Pp. iv+33–41. 15 cents. Fishery Bulletin 158: Food of the Squawfish *Ptychocheilus oregonensis* (Richardson) of the Lower Columbia River. By Richard B. Thompson. Pp. iv+43–58. 20 cents. Fishery Bulletin 159: Effects of Fertilizing Bare Lake, Alaska, on Growth and Production of Red Salmon (*O. Nerka*). By Philip R. Nelson. Pp. iv+59–86. 25 cents. Fishery Bulletin 160: Changes in Tuna Landings of the Hawaiian Longline Fishery, 1948–1956. By Richard S. Shomura. Pp. iv+87–106. 20 cents. (Washington, D.C.: Government Printing Office, 1959.) [3012

British Territories in Borneo: Geological Survey Department. Memoir No. 9: The Geology and Mineral Resources of the Sandakan Area and Parts of the Kinabatangan and Labuk Valleys, North Borneo. By Dr. F. H. Fitch. Pp. xvi+202+40 plates. (Kuching, Sarawak: Government Printing Office, 1958.) 6 Malayan dollars. [3012

Transvaal Museum, Pretoria. Report for the year ending 31st March, 1959. Pp. ii+48. (Pretoria: Transvaal Museum, 1959.) [3012

Die Bildung Organischer Meterie aus Kohlensäure. Von Prof. Dr. A. Eichinger. Studien über Stärkebildung Ausserhalb der Pflanze. Von Prof. Dr. A. Eichinger. (Bayreuth: Prof. Dr. A. Eichinger, Albert Preu-Strasse, 1, 1958 and 1959.) [3012

West African Maize Research Unit. Trial Report, 1957. Pp. 40. (Ibadan: West African Maize Research Unit, Moor Plantation, 1959.) [3012

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